

Annals of the Missouri Botanical Garden

Vol. 15

FEBRUARY, 1928

No. 1

A NEW GENUS OF THE ACANTHACEAE¹

CLARENCE EMMEREN KOBUSKI

Assistant, Arnold Arboretum of Harvard University

Formerly Rufus J. Lackland Research Fellow in the Henry Shaw School of Botany
of Washington University

A critical study of herbarium material of the genus *Dyschoriste* has revealed a small group of plants which possess sufficient morphological characters differing from *Dyschoriste* to merit generic recognition.

*Apassalus*² nov. gen. of the Acanthaceae. Calyx profunde 5-fidus. Corolla infundibuliforma; limbus subbilabiatus vel subaequalis; lobi rotundi, convoluti. Stamina 4, didynama, per paria lateraliter contigua vel connata decurrentia; antherae biloculares, basi obtusae, non acutae. Stigmatis lobus anticus obliquus vel dilatus, posticus subnullus. Capsula oblongo-linearis. Semina 2-4, plane compressa, suborbicularia.—Herbae perennes. Foliae ovatae, parvae. Flores parvae, solitarii vel in axillis fasciculati.

Type species: *Apassalus diffusus* (Nees) Kobuski.

KEY TO SPECIES

- A. Capsule 2-seeded; plants covered with short, hirsute, spreading hairs;
(Haiti).....*A. diffusus*
- AA. Capsule 4-seeded; plants glabrous.
 - B. Leaves 9-12 mm. long, ovate-subrotund; flowers 8-9 mm. long;
(Cuba).....*A. cubensis*
 - BB. Leaves 25-45 mm. long, ovate-elliptic; flowers 11-12 mm. long;
(Am. bor.).....*A. humistratus*

A. diffusus (Nees) Kobuski, n. comb.

Pl. 1, 2.

Dyschoriste diffusa (Nees) Urb. Symb. Ant. 7: 380. 1912.

¹ Issued April 30, 1928.

² Name dervied from the Greek α, *without* and πάσσαλος, *peg*, on account of the absence of anther appendages.

Dipteracanthus diffusus Nees in DC. Prodr. 11: 124. 1847.

Dyschoriste humistrata Lindau in Urb. Symb. Ant. 2: 188. 1900, not O. Ktze., namely, as to plants of Santo Domingo.

Stems somewhat tetragonal, slender, shortly hirsute, ascending from a perennial base, nodes closely placed, 1–2.5 cm. distant; leaves suborbicular-obovate, broadly obtuse at the apex, narrowing into a petiolate cuneate base, shortly hirsute on both surfaces, entire, 10–13 mm. long, 5–9 mm. wide; inflorescence bracteate, axillary; calyx 6–7 mm. long, lobes linear-acuminate, ciliate, $\frac{3}{4}$ total length; corolla white (ex Buch) or pale lilac (ex Tuerckheim), puberulent on the external surface, 7–8 mm. long, tube extending into a slightly amplified throat, lobes rounded; anthers didynamous, filaments slightly pilose at the base, anther cells parallel or nearly so, truncate or rounded at the base; ovary 2-celled, glabrous, style linear, pubescent a little above the base, stigma dilated, oblique; capsule 6–7 mm. long, 2-celled, each cell containing a single seed attached by the retinaculum, both of which (retinacula) are situated on the central ridge of the commissural surfaces; seeds flat, orbicular, becoming mucilaginous when wetted.

Distribution: Islands of Haiti and Santo Domingo.

Specimens examined:

Haiti: on rocky outcrop, dry wooded mountain slope, vicinity of St. Marc, 25–28 Feb. 1920, *E. C. Leonard* 2913 (US, G); dry bank along road near Ennery, Dept. of Artibonite, 325–900 m. alt., 13 Jan. 1926, *E. C. Leonard* 8823 (US); arid thickets, north-east of the N. West Indies Company, vicinity of St. Michel de l'Atalaye, Dept. du Nord, 300 m. alt., 17 Nov. 1925, *E. C. Leonard* 7093 (US); common in dry thickets, vicinity of St. Michel de l'Atalaye, Dept. du Nord, 350 m. alt., 26 Nov. 1925, *E. C. Leonard* 7472 (US); Barahona, 1200 m. alt., Sept. 1911, *Fuertes* 1407b (FM, G, US).

Santo Domingo: Azua, March, 1913, *Rose, Fitch & Russel* 4072 (US).

A. cubensis (Urb.) Kobuski, n. comb.

Pl. 1, 2.

Dyschoriste cubensis Urb. Symb. Ant. 7: 381. 1911.

Dyschoriste humistrata Lindau in Urb. Symb. Ant. 2: 188. 1900, not O. Ktze., namely, as to plants of Cuba.

Ruellia diffusa Grisebach, Cat. Pl. Cub. 195. 1866 (excl. syn.); Sauv. Fl. Cub. 97 (no. 1500). 1873.

Low-growing perennial, decumbent, occasionally rising erect, glabrous or minutely scabrous, young stems densely covered with cystoliths; leaves shortly petiolate, ovate to suborbicular, 9–12 mm. long, 5–7 mm. wide, rotund at the apex, tapering to a cuneate base, entire, densely covered with cystoliths on both surfaces, glabrous; flowers solitary, rarely in twos, bracts narrowly obovate; calyx 5-cleft, 6–8 mm. long, lobes linear-acuminate, nearly $\frac{3}{4}$ total length, entire external surface covered with cystoliths, glabrous, lobes ciliated; corolla 8–9 mm. long, tube cylindrical, enlarging until amplified throat is reached, lobes shortly obovate; stamens didynamous, adnate to the middle of the tube, anthers narrowly ovate, obtuse at the base; ovary 2-celled, style linear, nearly glabrous; capsule oblong-linear, 7–8 mm. long, glabrous, 4-seeded; seeds suborbicular, mucilaginous when wetted.

Distribution: near Cojimar, Prov. of Havana, Cuba.

Specimens examined:

Cuba: near Cojimar, Prov. of Havana, 14 March, 1906, *Baker 2894* (FM); shady places in coastal sand between Rio Cojimar and Playa de Bacuranao, Prov. of Havana, 26 Dec. 1910, *Wilson 9533* (G, US).

A. humistratus (Michx.) Kobuski, n. comb. Pl. 1

Dyschoriste humistrata (Michx.) O. Ktze. Rev. Gen. Pl. 2: 486. 1891.

Ruellia humistrata Michx. Fl. Bor.-Am. 2: 23. 1803; Pursh, Fl. Am. Sept. 2: 421. 1814.

Calophanes humistrata Shuttleworth ex. Nees in DC. Prodr. 11: 108. 1847; Gray, Syn. Fl. N. Am. ed. 1, 2¹: 324. 1878, and ed. 2. 1886; Chapman, Fl. Southeastern U.S. ed. 1, 1083. 1860, and ed. 2. 1889.

Dipteracanthus humistratus Chapman, Fl. Southeastern U.S. ed. 2, 303. 1889.

Dipteracanthus riparius Chapman, Fl. Southeastern U.S. ed. 2, 303. 1889.

Stems several, ascending or rising erect from a ligneous perennial base, 4 dm. or less high, glabrous or slightly pubescent;

leaves ovate-elliptic to oblong-sublanceolate, 2.5–4.5 cm. long, 1–2 cm. broad, obtuse to acute at the apex, abruptly attenuated at the base into a petiole which may be so short as to give the leaf a sessile appearance or as much as 4 mm. long, glabrous or nearly so, entire or slightly crenulate margins; bracts oblong-ob lanceolate, about equalling the length of the flower; flowers axillary; calyx deeply 5-parted, 9–10 mm. long, glabrous or slightly pubescent, lobes subulate-setaceous; corolla small, white, 10–11 mm. long, tube 2.5–4 mm. long; stamens didynamous (very seldom 5), filaments pubescent at point of adnation to corolla throat, anther cells obtuse or slightly mucronulate at the base; mature capsule 9–10 mm. long, glabrous, linear, 4-seeded.

Distribution: low grounds, southeastern United States.

Specimens examined:

Georgia: Lumber City on the Ocmulgee River, Telfair Co., July, 1900, *C. Mohr* (US, 721392); shaded places in Ogeechee River swamp, Burke Co., 5 June, 1901, *R. M. Harper* 769 (M, US).

Florida: fertile ground under oaks, upper St. John's River, 1 June, *A. H. Curtiss* 23 (G); Hot Springs, 7 April, 1925, *H. O'Neill* 601 (US); Pine Island, St. John's River, 11 April, 1911, *S. C. Hood* (G); swampy shore of St. John's River, June, 1878, *A. H. Curtiss* 1939 (M, FM, G, US); wooded banks of the Suwannee River at Branford, Suwannee Co., 9 June, 1900, *A. H. Curtiss* 6654, (G, M); Suwannee Co., June–July, 1898, *A. S. Hitchcock* 1457, 1458 (FM); damp shady places, banks of Rice Creek, Putnam Co., 26 March, 1882, *C. Mohr* (US 721391); Dunnellon, Marion Co., 25 Feb. 1891, *L. F. & R. Ward* (US, 147428); Port Orange, Volusia Co., 20 May, 1895, *F. C. Straub* 164 (G); Lake Alfred, Polk Co., 11 June, 1922, *G. M. & J. K. Armstrong* (M 911680); swamp, Hernando Co., June–July, 1898, *A. S. Hitchcock* (M 120820).

THE JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION	
PUBLISHED WEEKLY	
CHICAGO, ILL., MAY 1, 1914	
CONTENTS	
ORIGINAL ARTICLES	1
REPORTS	1
EDITORIALS	1
DEPARTMENTS	1
NOTES	1
LETTERS	1
ADVERTISEMENTS	1
INDEX	1

EXPLANATION OF PLATE

PLATE 1

Apassalus diffusus (Nees) Kobuski

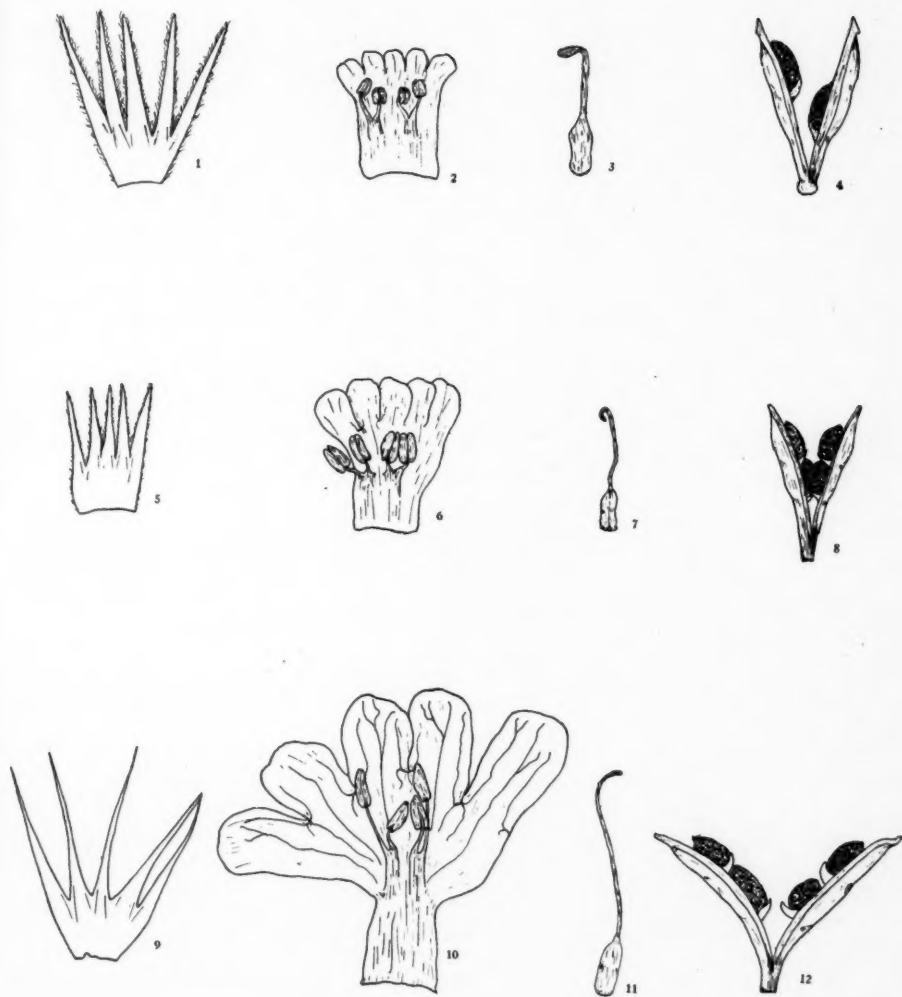
- Fig. 1. Open calyx.
Fig. 2. Open corolla showing stamens.
Fig. 3. Pistil.
Fig. 4. Dehiscing capsule showing seeds and retinacula.

Apassalus cubensis (Urban) Kobuski

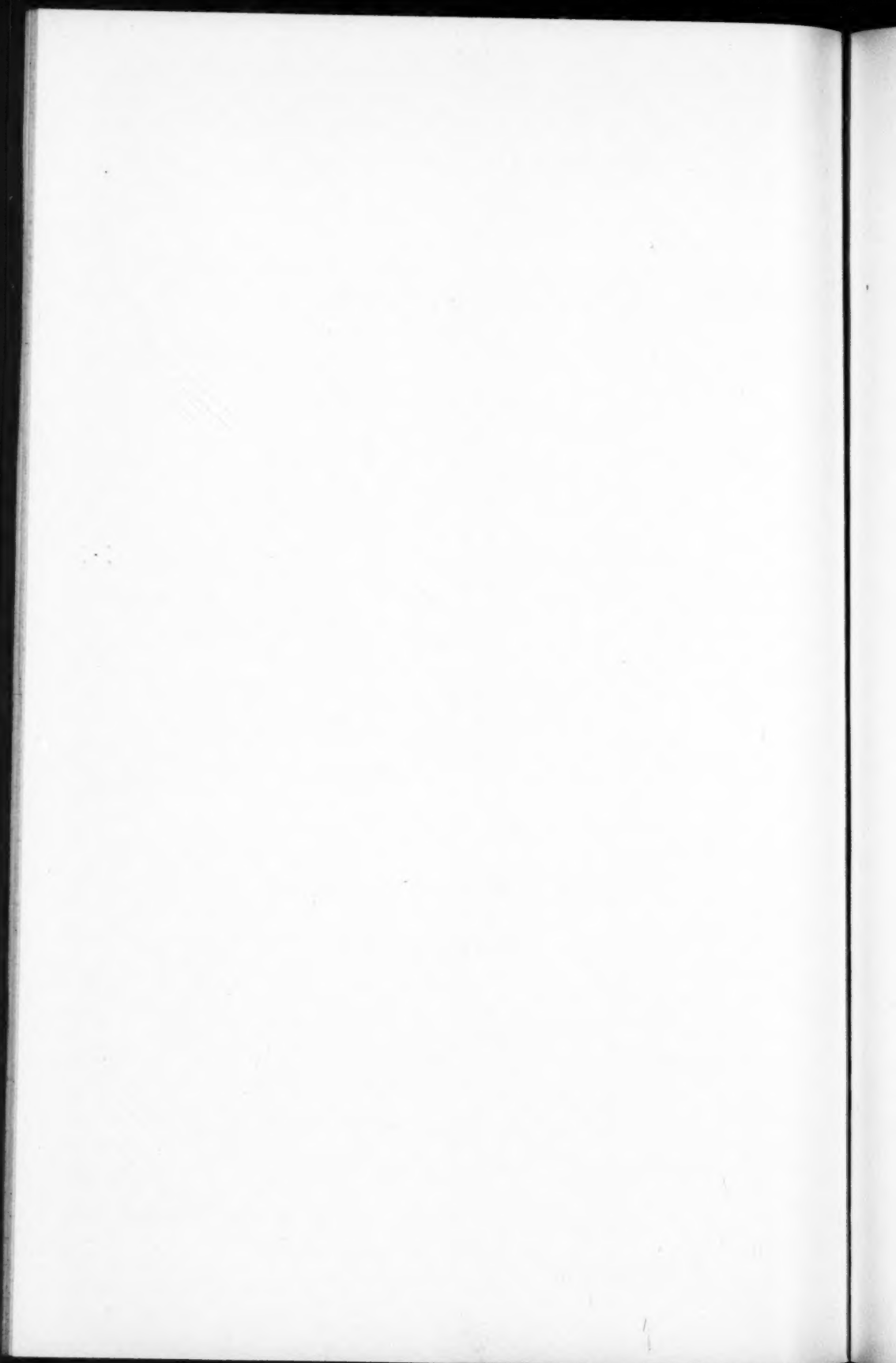
- Fig. 5. Open calyx.
Fig. 6. Open corolla showing stamens.
Fig. 7. Pistil.
Fig. 8. Dehiscing capsule showing seeds and retinacula.

Apassalus humistratus (Michx.) Kobuski

- Fig. 9. Open calyx.
Fig. 10. Open corolla showing stamens.
Fig. 11. Pistil.
Fig. 12. Dehiscing capsule showing seeds and retinacula.



KOBUSKI—A NEW GENUS OF ACANTHACEAE



EXPLANATION OF PLATE

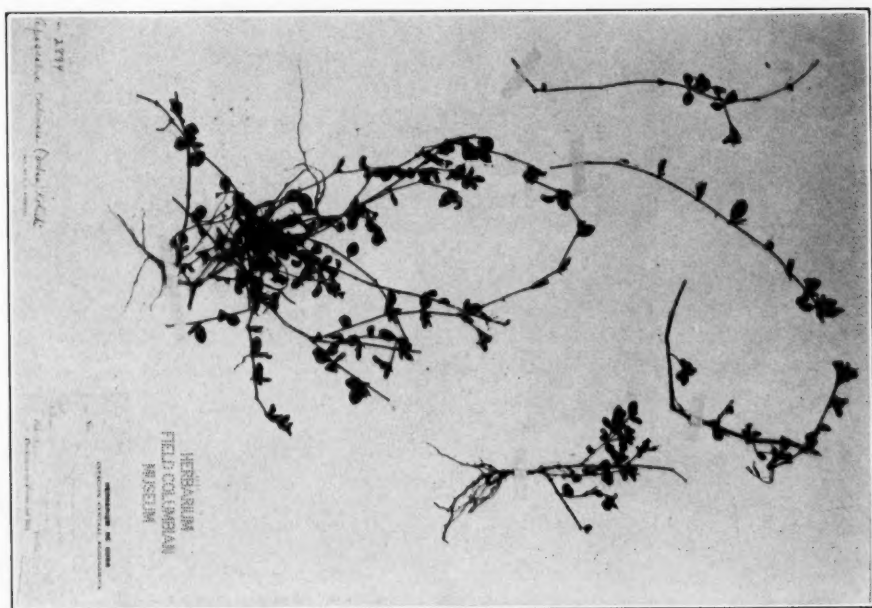
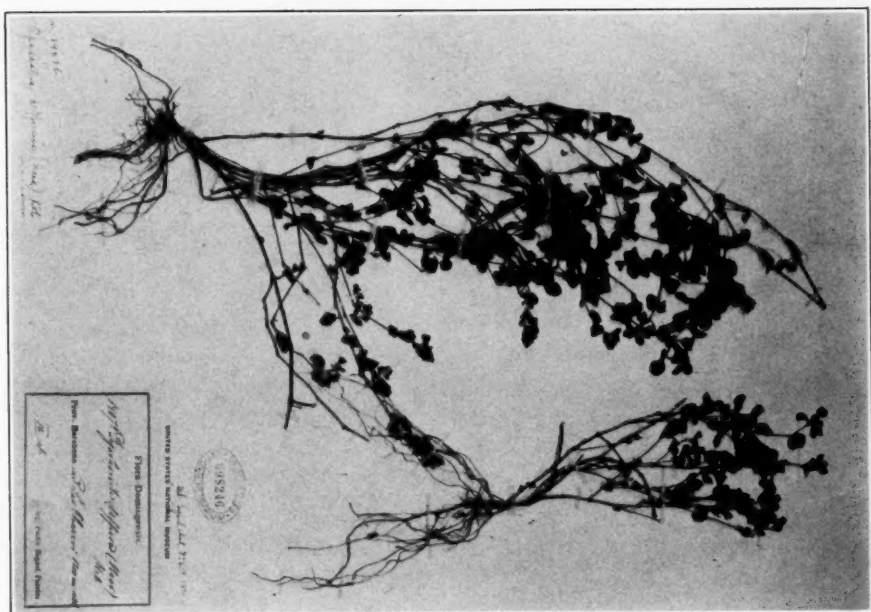
PLATE 2

Fig. 1. *Apassalus diffusus* (Nees) Kobuski

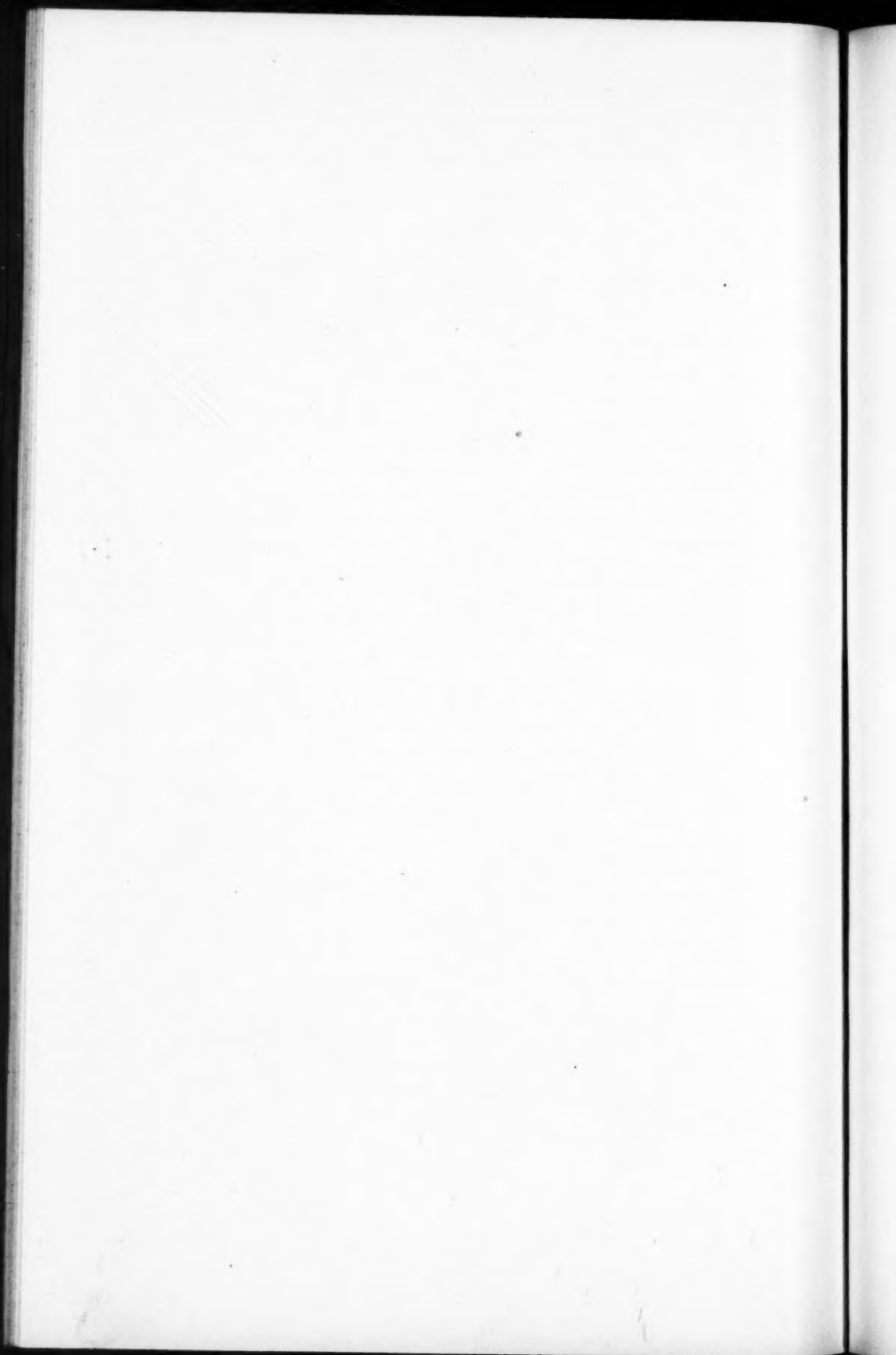
From the specimen, *Fuertes 1407b*, in the United States National Herbarium,

Fig. 2. *Apassalus cubensis* (Urban) Kobuski

From the specimen *Baker 2894*, in the Herbarium of the Field Museum.



KOBUSKI—A NEW GENUS OF ACANTHACEAE



A MONOGRAPH OF THE AMERICAN SPECIES OF THE GENUS *DYSCHORISTE*¹

CLARENCE EMMEREN KOBUSKI

*Assistant, Arnold Arboretum of Harvard University
Formerly Rufus J. Lackland Research Fellow in the Henry Shaw School of Botany of
Washington University*

INTRODUCTION

Any attempt to determine specifically herbarium specimens of the genus under present consideration formerly proved very unsatisfactory because of the inadequacy of many of the original descriptions and also because of the small representation of type or authentic material in American herbaria. These facts, along with an especial interest in the genus and its allies, led to the present study. At first it was hoped that a monographic treatment of the whole genus might be made. A general survey of the material deposited in American herbaria, however, showed the Old World species to be so poorly represented that it was deemed advisable to exclude them from the present discussion and to include only the American species. Later the writer plans to visit some of the larger European herbaria and to supplement this monograph by a critical study of the far-eastern species.

This investigation was made possible only through the coöperation of the botanists connected with the various herbaria from which material was borrowed. Sincere appreciation is due Dr. B. L. Robinson of the Gray Herbarium, W. R. Maxon of the United States National Herbarium, and D. C. Davies, Director of the Field Museum, who so willingly loaned their entire collections of *Dyschoriste* for this study. It was found necessary also to borrow types, and to obtain fragments and photographs of type collections from several European herbaria. Dr. Santiago Ramón y Cajal, Instituto Cajal, Madrid, and Professor Eduardo Balguerías y Quesada, Jardín Botánico, Universidad de Madrid,

¹ An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

Issued April 30, 1928.

very kindly furnished an excellent photograph of a little-known species, the type of which is preserved in the Madrid Herbarium. Professor Boris Fedtschenko, Jardin Botanique Principal, Lenin-grad, U.S.S.R., obligingly supplied two types essential for the completion of this monograph. Dr. A. W. Hill and T. A. Sprague, Royal Botanic Gardens, Kew, Dr. L. Diels, Botanischer Garten und Museum zu Berlin-Dahlem, Dr. C. H. Ostenfeld and Dr. Carl Christensen, Botanisk Garten, Københavns Universitet, as well as Dr. Adele Lewis Grant, Huguenot College, South Africa, who so willingly made critical comparisons with types at the Kew Herbarium on her journey to Africa, have all contributed either directly or indirectly, in material loaned or in verification of specimens submitted for comparison. The writer takes this opportunity to express his gratitude for their generosity and kindly assistance.

This study was made at the Missouri Botanical Garden, and thanks are due to the Director, Dr. George T. Moore, for the use of the excellent library and herbarium facilities which this institution affords. Also, especial thanks are extended to the Curator of the Herbarium, Dr. Jesse M. Greenman, under whose constant guidance and supervision this work has been made possible.

HISTORY

The genus *Dyschoriste* was proposed by Nees in the third volume of Wallich's 'Plantae Asiaticae Rariores' ¹ published in 1832. The genus was segregated from *Ruellia* on account of stamen, corolla, and fruit characters, and was based on *Ruellia depressa* Wallich, namely, Wallich's No. 2379 from East India, which, however, is not conspecific with *Ruellia depressa* L. Two other East Indian species, *D. cernua* and *D. litoralis*, were referred by Nees in the same work to his new genus.

In 1833, only a year later, David Don in Sweet's 'British Flower Garden' ² described the genus *Calophanes*. Don's new genus was also a segregate from *Ruellia*, and was founded on *Ruellia oblongifolia* Michaux, which in turn was based on specimens collected in the state of Georgia.

¹ Wallich, N. Pl. As. Rar. 3: 81. 1832.

² Sweet, R. Brit. Fl. Gard. II, 2: 181, pl. 181. 1833.

These two generic names were current in botanical literature for many years, as representing two supposedly distinct genera indigenous to remote regions—*Dyschoriste* of the eastern hemisphere and *Calophanes* of the western hemisphere. Nees, the foremost student in his time of the Acanthaceae, in his treatment of this family for Martius' 'Flora Brasiliensis,'¹ in 1847, accepted *Calophanes* and described seven Brazilian species of this genus. The same author and in the same year elaborated the Acanthaceae for DeCandolle's 'Prodromus,'² and maintained both names as representing separate and distinct genera. In this work, which was the first to present a comprehensive treatment of the group, five species of *Dyschoriste* and twenty-seven species of *Calophanes*, as well as several varieties, were recognized.

Bentham and Hooker in the 'Genera Plantarum,'³ 1876, treated these two previously supposed distinct generic elements as congeneric, but unfortunately they took up the later name *Calophanes* and relegated *Dyschoriste* to synonymy.

Mr. C. B. Clarke, who contributed the treatment of the Acanthaceae for Hooker's 'Flora of British India,'⁴ 1885, followed Bentham and Hooker's generic interpretation of the group and recognized four East Indian species of *Calophanes*, namely, *C. Nagchana* Nees, *C. littoralis* T. Anders., *C. vagans* Wight, and *C. Dalzellii* T. Anders. Under *C. Nagchana* Nees the following species are cited as synonyms: *C. depressa* T. Anders., *Ruellia Nagchana* Ham., *R. erecta* Burm., *R. depressa* and *R. cernua* Nees, *Dipteracanthus Nagchana* Nees, *Dyschoriste depressa*, and *D. cernua* Nees.

In 1891, Dr. O. Kuntze⁵ revived the name *Dyschoriste* Nees and transferred thereto several species, including *Ruellia erecta* Burm. which was described and illustrated in 1768, being the oldest known species of this group. Lindau, in 1895, in reviewing the Acanthaceae for Engler and Prantl's 'Die Natürlichen Pflanzenfamilien,'⁶ followed Kuntze in recognizing the genus *Dyschoriste*.

¹ Martius, C. F. P. de. Fl. Bras. 9: 26. 1847.

² DeCandolle, A. P. Prodr. 11: 106, 107. 1847.

³ Bentham, G. & Hooker, J. D. Gen. Pl. 2: 1077. 1876.

⁴ Hooker, J. D. Fl. Brit. India 4: 410. 1885.

⁵ Kuntze, O. Rev. Gen. Pl. 2: 486. 1891.

⁶ Engler, A. & Prantl, K. Nat. Pflanzenfam. 4th: 302. 1895.

Clarke, in working up the Acanthaceae for the 'Flora Capensis,'¹ 1901, took up the name *Dyschoriste*, thus reversing the position taken by him in Hooker's 'Flora of British India,' mentioned above. He recognized *D. depressa* Nees as a valid species along with four other African species, one of which is *D. erecta* Clarke, thus apparently disregarding the *D. erecta* (Burm.) O. Ktze.

Several new species have been described from time to time and referred to either *Dyschoriste* or *Calophanes*, but no comprehensive treatment of the group as a whole has been published since that of Nees in DeCandolle's 'Prodromus.'

Assuming that there is absolute identity and thus complete synonymy of the several elements which Clarke referred to *Calophanes Nagchana* in the 'Flora of British India,' then, as pointed out by Dr. Kuntze, the name *erecta*, as the oldest specific name involved, must be retained and the binomial *Dyschoriste erecta* (Burm.) O. Ktze. becomes the valid combination for the plant concerned and *D. depressa* (Wall.) Nees must be regarded as a synonym of it. Since this study has been confined to the American species we must admit the observations of Clarke and Kuntze and accept *Dyschoriste erecta* (Burm.) O. Ktze. as the type species of the genus until the eastern species in question can be examined.

GENERAL MORPHOLOGY

Roots.—The root system in the genus *Dyschoriste* is not very extensive. All the species are perennial and the roots, in turn, are of the simple fibrous form. By the casual observer, however, some of the slender underground stems of the previous year's growth are sometimes mistaken for roots.

Stems.—There is considerable variation in the stem and its habit of growth. In all cases, the stem or stems arise from a ligneous perennial base. However, the mode of ascent varies. Many species are prostrate and the stems spread over the ground in several directions. In these cases the leaves assume a secund position. Often when the stems are short a rosette appearance is attained for the whole plant. The species *D. oaxacensis* illus-

¹ *Flora Capensis* 5: 15. 1901.

trates this character. However, it is not a stable specific characteristic. A second habit of growth is the ascending type. It is in this category that the majority of species is placed. The stem not infrequently becomes more or less geniculate; this mode of growth is very characteristic of *D. Pringlei*. The third habit of growth is the erect type. It is to this type that the sturdy *D. hirsutissima*, *D. oblongifolia*, *D. ovata*, and *D. trichanthera* belong. Some species may follow consistently a distinct habit of growth, while others may have the stems ascending or erect even on the same plant. One can usually associate stem growth exceeding a length of 4–5.5 dm. with the erect habit, and shorter stems with the ascending or prostrate habit. Along with this, the prostrate type will possess small leaves and the erect type will have leaves with a more extensive surface.

Several species, among them *D. oblongifolia*, possess slender underground stems which are common in perennials. These stems have the appearance of roots but on close observation buds and modified leaves can be seen. After passing under the ground for some distance they come to the surface and then rise erect.

The stem may be terete or quadrangular. The latter is the more common type in the genus. In the species *D. quadrangularis* the stem is not only angular but the angles are winged. This condition is probably brought about by the decurrent petiole of the leaf extending down the stem.

Leaves.—The leaves of the species in the genus *Dyschoriste* present a great variety of differences. All species have leaves with entire margins, except *D. bilabiata* which is not distinctly dentate but has a decided tendency in that direction. Several species, such as *D. crenulata* and *D. hirsutissima*, show a tendency toward crenulate margins. Others combine the crenulate with the repand margin. Along with these characteristics the margin is usually ciliate. In shape the leaf varies from that of the narrow, linear *D. angusta*, *D. Greenmanii*, and *D. Purpusii* to that of the oblong-ovate *D. quadrangularis* which grows to a length of 10 centimeters. Some species have two types of leaves, the lower or cauline leaves often being larger and different in shape from the upper leaves in whose axils branches and flowers are crowded.

The surface of the leaf itself is usually pubescent. When the pubescence is sparse or absent, an abundance of cystoliths is usually seen. Often the cystoliths, since they lack an orderly arrangement, are mistaken for appressed hairs. This cystolithic character is not sufficiently definite for specific delimitation. Some plants have a more copious formation of cystoliths than others. This character exists on the stem and calyx as well as the leaves. The venation which is very pronounced on the lower surface varies very little within the genus. The usual form is the feather-veined type.

Inflorescence.—In all cases the flowers are axillary and subtended by bracts and sometimes bracteoles. Only occasionally are the flowers solitary in the axils. There are usually several to many flowers at the node, giving the appearance of a cymose cluster as in *D. quadrangularis* or a capitulum as in *D. capitata* and *D. pinetorum*. *D. Greenmanii* is an excellent example of a species with a solitary flower at the node. The majority of species have flowers which are pedicellate, often so short, however, that a sessile effect is presented.

Calyx.—In the calyx of *Dyschoriste* is found one of the most constant characters of the genus. It is usually five-parted and always persistent. The only deviation from the five-merous condition is found in *D. maranhonis* where the calyx-lobes are occasionally only four. In all cases the lobes are subulate-setaceous and usually ciliate. The ciliation may vary from a long whitish, flaccid pubescence to a very short hirsuteness. When the calyx proper is pubescent, the pubescence is usually confined to the nerves. The tissue connecting the lobes of the calyx is usually very membranaceous and tears apart very easily, making it difficult and quite unsatisfactory to use the ratio between tube-length and lobe-length as a character for specific differentiation. The lobes are usually quite equal in length. However, here again variation is found. Cystoliths, as in the leaves, are very abundant; but in the calyx they are frequently disposed in a more or less regular arrangement.

Corolla.—There is very little differentiation to be found in the corolla of the genus. In most cases the bilabiate type occurs. This, however, is not as distinct as in some other genera of the

Acanthaceae. The corolla is five-lobed, the two posterior lobes being coalescent to a greater extent than the three anterior lobes. The length of the corolla varies from 10 to 17 mm., as found in *D. decumbens*, *D. hygrophiloides*, *D. saltuensis*, *D. quadrangularis*, and *D. angustata*, to 25–28 mm., exemplified in *D. xylopoda*, *D. humilis*, and *D. ovata*. One species, *D. Pringlei*, has a corolla measuring 35–38 millimeters long. None, however, reach the length of 70–80 millimeters as found in some of the Ruellias. The proportion between tube and throat is variable in the genus. The ventricose throat is found quite often. The condition should exist in all species because of the contiguity of the adnate filaments in the posterior portion of the throat and tube. This ventricosity, hence, is more pronounced in the larger-flowered species. The narrow tube of the corolla is usually slightly flared at the base to make room for the disc and ovary. The ampliation from the tube to the throat is very variable and may be abrupt or gradual according to the species. In all cases, the external surface is quite pubescent. In the species *D. trichanthera* the pubescence is found on the interior as well as the exterior surface of the throat.

Stamens.—The stamens are didynamous. The long and short filaments on each side are contiguous or united at the base by a membrane which extends from the point of adnation to the base of the corolla tube. A very distinctive feature of the anthers is the mucronate appendages at the base of the anther cells. These mucronate appendages are characteristic of the genus and are very easily seen with the hand-lens. Under the low power of the compound microscope they are found to be composed of several multicellular strands of cells closely compacted together. These strands of cells are easily torn apart, and a dentate or ragged appearance is given to the whole appendage. This is doubtless what Nees saw when he described the appendages of *D. quitensis* as "2-3-toothed." A similar example was found in *D. Schiedeana*. On microscopic study, however, the so-called dentations were found to be nothing more than shreds of tissue torn away slightly from the compacted mass. The anther cells are usually parallel and oblong in shape. In the case of *D. sagittata* and *D. maranhonis* the cells are so disposed as to have a

sagittate appearance. In both the species mentioned the apex as well as the base is appendaged. As a rule the anther cells are glabrous but in the species *D. trichanthera* the anther cells are very pubescent. The mode of dehiscence is by a longitudinal slit on the side of the anther cell. The filaments are commonly pubescent.

Pistil.—There is little variation in the parts of the pistil. A disc is present beneath the ovary in all species. The ovary itself is two-celled, glabrous, and oblong. Little or no variation is found in the filiform pubescent style. Only the anterior lobe of the stigma is developed, and this lobe is usually linear and oblique with a flattened stigmatic surface. However, in *D. hygrophiloides* the stigma is curved, while in *D. sagittata* it is basally lobed. In *D. maranhonis* the stigma is reflexed.

Capsule.—The capsule of the genus is quite uniform. The constant linear, glabrous and four-seeded characters, combined with other diagnostic characters, help considerably in generic determination. Retinacula or hooked appendages on the median ridge of the valves hold the flat suborbicular seeds in place. When dry the seeds appear to have many soft, appressed hairs. These same hairs when wetted diverge, elongate, and become mucilaginous.

GEOGRAPHICAL DISTRIBUTION

The geographical distribution of the American species of the genus *Dyschoriste* offers very interesting problems. The accompanying maps demonstrate very clearly that there are three distinct areas of distribution: (1) southeastern United States; (2) southwestern United States and Mexico; and (3) South America.

Two species, namely, *Dyschoriste oblongifolia* and *D. angusta*, occur in the southeastern United States area. This area extends the width of the coastal plain from southern Virginia to southern Florida. The regions of distribution of the two species do not overlap. *D. angusta* is confined to the wet region of Dade and Palm Beach Counties in southern Florida, while *D. oblongifolia* extends northward through the remainder of the area, seeking the dry, sandy pine woods.



Fig. 2. Map showing the geographical distribution of the South American species of *Dyschoriste*.

to this area. It is an interesting fact that the distribution of some species is almost coincident with the geological formation of the country. *D. decumbens*, which occurs on the plateau

region between the Sierra Madre ranges, is an excellent example of this fact. *D. hirsutissima* extends from Sonora southward along the western slope of the Sierra Madre range to Oaxaca. Many species have a localized distribution only, *D. Greenmanii*, *D. crenulata*, *D. saltuensis*, and *D. angustifolia* being examples. Some of these localized areas are characterized by three or more species. An instance of this is a small region around Guadalajara in the state of Jalisco where four species are represented. Another illustration of limited areal distribution occurs in the northern part of Oaxaca which harbors *D. oaxacensis*, *D. angustifolia*, *D. capitata*, and *D. hirsutissima*. Many species, especially the localized ones, appear to be extremely edaphic since they inhabit only regions near volcanoes. The center of distribution in this second area falls within the region represented by the states of Puebla, Michoacan, and Mexico.

The third and last area, namely that of South America, comprises more territory than either of the areas indicated above and includes the seventeen remaining species of the genus. The material examined in all cases was not very copious, hence an accurate range of geographical distribution of these species could not definitely be ascertained. Nearly all species appear to occur in isolated and limited areas, but the relationship between some of these areas indicates that a greater overlapping of areas would occur were it not for the paucity of herbarium material. Three species, namely, *D. quitensis*, *D. ciliata*, and *D. repens*, are found in Peru and the Andes of Ecuador. *D. Niederleinii* and *D. humilis* are found in Argentina. The other thirteen species inhabit northern Paraguay and southern Brazil, and it is here that the South American center of distribution occurs.

An unusual feature of the geographical distribution is the isolation of the areas defined. At present, there is no one species which connects up any two areas. There seems to be no satisfactory explanation to account for the absence of the genus between the Andes of Ecuador and the Isthmus of Tehuantepec in Mexico. Members of the genus may be found between these two remote regions, but until the entire Andean range has been explored more thoroughly from a botanical standpoint one would hardly venture an explanation of the marked discontinuous distribution which the genus now presents.

The non-occurrence of the genus in the Mississippi Valley is equally surprising. Since the flora of this entire area is comparatively well known, it is hardly possible that the members of the genus would be overlooked if they there existed. The only solution seems to be the possible age of the genus. *Dyschoriste* is probably a pre-glacial genus which, prior to the Oligocene period of the Cenozoic era, extended continuously across the southern United States. However, the Eocene and Oligocene seas encroached upon the United States in the present Mississippi Valley, thereby splitting the distribution areas of the genus into two parts.

PHYLOGENY

Because of the large number of closely allied species in the genus *Dyschoriste* it is quite necessary that the phylogenetic discussion of the group be made from a purely hypothetical standpoint. The fact that the discussion is confined to the American species alone seconds this consideration, since the eastern species of the genus exceed the American species in number.

On account of the three distinct geographical distribution areas, which have been discussed before, a tree method of illustrating probable phylogenetic sequence proved unsatisfactory; hence the method used in the accompanying chart was devised finally to illustrate the apparent relationship of the species of the western hemisphere. This chart if superimposed on a map of the regions inhabited by the genus would coincide with the specific regional distribution.

It was felt reasonably certain that all species of the genus have evolved from a common ancestor designated in the chart as x . From this ancestor, species and groups of species have evolved. One might ask why, since the species seem to be placed in definite groups, subgenera or sections have not been designated. This question was given much thought and consideration; it was felt finally, however, that on account of the relative uniformity of the essential morphological characters within the genus, except in the case of group II, no adequate basis exists for the designation of subgenera or sections.

It may be observed that all the designated groups with the

exception of group II originated from the common ancestor at approximately the same time. Group II, on account of the muticous character of its anther appendages, the extremely small leaves, the very small flowers, and the fruit characters, has been separated from the genus *Dyschoriste* and raised to the rank of a new genus which is considered as intermediate between the hypothetical type and *Dyschoriste* proper. In this genus, *Apassalus*,¹ containing three species, the species *Apassalus diffusus*

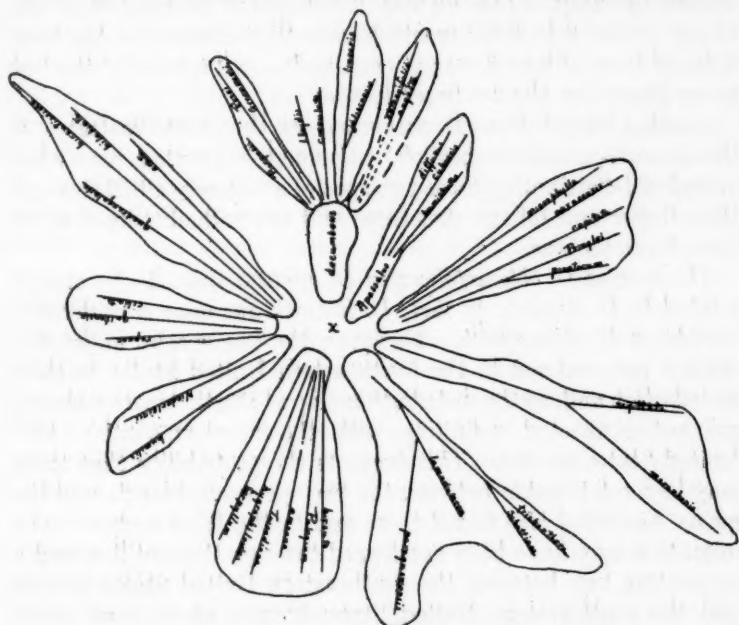


Fig. 3. Phylogenetic chart of the species of *Dyschoriste*.

has reached the highest development in the reduction of the number of ovules to two, one being borne in each valve of the capsule. The other two species contain the four seeds which are characteristic of the majority of species in the genus *Dyschoriste*. *Apassalus* is confined to the islands of Cuba and Haiti and to the southeastern United States.

¹See Kobuski, C. E. A new genus of the Acanthaceae. Ann. Mo. Bot. Gard. 15: 1-8, pl. 1-2. 1928.

Group I, involving ten species, is confined to the plateau regions of Mexico. In this instance *D. decumbens*, on account of its extended range and characteristic relation to all species concerned, is considered the base species. *D. Lloydii* and *D. crenulata* are species having vital characteristics similar to *D. decumbens* but differing sufficiently in minor characters to be considered direct descendants from the base species. A small group containing three species, *D. Purpusii*, *D. Greenmanii*, and *D. Rosei*, stands by itself. The highest development is reached in *D. Greenmanii* and *D. Rosei* in which cases the inflorescence has been reduced to a solitary flower at each node. All species of the last group have very slender linear leaves.

Another branch from *D. decumbens*, as the chart illustrates, is the *linearis-jaliscensis* branch. Although separated somewhat in regional distribution these two species are closely allied through their flower and foliage structures and are undoubtedly derived from *D. decumbens*.

The species *D. oblongifolia* and *D. angusta* seem to be closely related to *D. linearis*; in fact, *D. linearis* was once considered a variety of *D. oblongifolia*. However, the two species under discussion are confined to the southeastern United States in their distribution and, as the distribution map of North America shows, are not connected definitely with the Mexican-southwestern United States species. This suggests the probability that there may be a relationship between the two species in Florida and the genus *Apassalus* also found there and in the West Indies. The migration may have been northward through the Antilles, and a connecting link between the southeastern United States species and the southwestern United States-Mexico group may never have existed.

In group III, one finds an entirely different situation. Here we have four large, erect species, each showing a definite characteristic development toward advancement. Perhaps the highest development is found in *D. hirsutissima* which extends the length of the western Sierra Madre range and possesses a well-developed glandular pubescence. This is the only instance of this character in the whole genus. *D. bilabiata* and *D. quadrangularis* are close relatives but do not seem to have been derived from the *D. hir-*

sutissima line. Instead, they undoubtedly arose along parallel lines of development. In *D. bilabiata* we find a distinct dentation of the leaf. This group is also characterized by large, petiolate leaves, in some cases as long as ten centimeters, an unusual feature in the genus.

Group IV for the most part occurs in southwest Mexico, that is, the states of Oaxaca, Jalisco, and Michoacan. These species are the ascending foliose type with rather small, ovate leaves. The inflorescence is usually subcapitate, and it is sometimes difficult to distinguish off-hand the species here included. They all, with the exception of *D. pinetorum*, seem to have evolved along a parallel line of development. *D. pinetorum*, on account of its resemblance to *D. Pringlei*, undoubtedly evolved from it. It is in *D. Pringlei* that we find the largest flower of the genus *Dyschoriste*, and in this species a close resemblance is shown to the genus *Ruellia*. The highest point of development in the North American species just discussed is found, according to my opinion, in *D. hirsutissima*, *D. Greenmanii*, *D. Rosei*, and *D. linearis*.

In the South American species of the genus, a similar situation is found. Here the species can be placed in five groups. Group V contains the three species on the western coast inhabiting Peru and Ecuador. Specialization in them is not particularly noticeable. The species *D. ciliata* possesses rather muticous anther appendages. This would ally the species to the genus *Apassalus*. It was necessary to accept the word of Nees in this instance, however, as only a photograph of the type of the species could be obtained.

The species of Group VI are found in Argentina. Here *D. humilis* reaches the highest point of development in the reduced number of seeds. As in *Apassalus diffusus*, only two seeds are produced, a single seed occurring in each valve of the capsule.

Group VII is not unusual in its development. In this group are found six closely related species—perhaps interrelated—but showing no special development. Here again the herbarium material at hand is very sparse, the study in a few instances being confined to photographs.

In Group VIII are four species, two of which are described for

the first time. In the species *D. trichanthera* is found the spicate inflorescence along with pubescent anthers. The former character links it up with *D. Schottiana*, while the latter character shows the relationship which exists between *D. trichanthera* and *D. lavandulacea*. *D. paraguariensis* is undoubtedly a branch from *D. lavandulacea* but possesses more highly developed floral characters.

The last group, namely, group IX, includes only two species. It is here that the sagittate or divergent anther cells are found. The highest development of the group and probably the highest development in the genus is shown by *D. maranhonis*, in which both incomplete didynamy and reduction in corolla and calyxlobes are found. Nees describes *D. maranhonis* as being glandular-pubescent. A fragment of the type specimen was obtained and failed to demonstrate this character.

SUMMARY

The conclusions drawn as to the probable phylogeny of the group under consideration are reached after a comparative study of the outstanding morphological characters which may be summarized as follows:

- (1) Muticous appendaged anthers are more primitive than those with apiculate appendages.
- (2) Divergent anther cells are more advanced than parallel anther cells.
- (3) Glabrous anthers are more primitive than pubescent anthers.
- (4) Four-seeded capsules are more primitive than two-seeded capsules.
- (5) Numerous flowers in an axil is a more primitive condition than the solitary-flowered axil because the presence of bracts in the solitary-flowered species shows reduction to have taken place in the telescoping of the inflorescence.
- (6) An unlobed stigma is more advanced than the lobed stigma.
- (7) Entire-margined leaves are primitive. The dentate margin is an advancement, and the crenulate margin type is intermediate.
- (8) Glandulosity is more advanced than pilosity.

(9) Procumbent plants are more primitive than erect plants which have evolved through ascending plants.

(10) Complete didynamy is more primitive than incomplete didynamy.

(11) Winged stems are more advanced than the unwinged quadrangular stems.

(12) Reduction of corolla- and calyx-lobes from five to four is a criterion of specialization and advancement.

ABBREVIATIONS

The abbreviations used to indicate the herbaria in which the specimens cited in the present paper occur are as follows:

B = Botanischer Garten und Botanisches Museum, Berlin, Germany.

C = Botanisk Garten, Københavns Universitet, Copenhagen, Denmark.

Ch = University of Chicago (deposited in the Field Museum).

FM = Field Museum of Natural History.

G = Gray Herbarium of Harvard University.

K = Royal Botanic Gardens, Kew, England.

L = Jardin Botanique Principal, Leningrad, U.S.S.R.

M = Missouri Botanical Garden.

Ma = Jardin Botanico, Universidad de Madrid, Madrid, Spain.

US = United States National Herbarium.

TAXONOMY

Dyschoriste Nees in Wallich, Pl. As. Rar. 3: 75, 78. 1832; Nees in DeCandolle, Prodr. 11: 106. 1847; O. Kuntze, Rev. Gen. Pl. 2: 485. 1891; Lindau in Engl. & Prantl, Nat. Pflanzenfam. 4^{3b}: 302. 1895; Gray, Manual, ed. 7, 743. 1908.

Calophanes D. Don in Sweet, Brit. Fl. Gard. 2: pl. 181. 1833; Nees in Mart. Fl. Bras. 9: 25. 1847; Nees in DC. Prodr. 11: 107. 1847; Benth. & Hook. Gen. Pl. 2: 1077. 1873-76; C. B. Clarke in Hooker, Fl. Brit. India 4: 410. 1885; Gray, Syn. Fl. N. Am. 2: 324. 1878, and ed. 2, 1886; Chapman, Fl. Southeastern U.S., ed. 3, 365. 1897; Small, Fl. Southeastern U.S. ed. 1, 1082. 1903, and ed. 2, 1913.

Linostylis Fenzl. in *Linnaea* 23: 94. 1850.

Herbaceous, caulescent perennials, prostrate, ascending or erect, glabrous or pubescent. Leaves opposite, sessile or petioled, usually entire. Inflorescence cymose, capitate or spicate, terminal or axillary. Flowers subtended by foliaceous bracts and bracteoles. Calyx deeply 5-lobed, lobes usually subulate-setaceous, ciliate, lineolate. Corolla-tube usually erect, occasionally slightly amplified at the base; limb spreading, oblique, obscurely or distinctly bilabiate, 5-lobed. Stamens 4, didynamous; filaments of a long and short stem united at the base and adnate to the base of corolla-tube, pubescent; anther 2-celled, cells oblong, sharply mucronate at the base, parallel or slightly divergent, glabrous or occasionally pubescent. Ovary 2-celled, glabrous, ovules 2 or occasionally 1 in each cell; style filiform, pubescent; posterior lobe of stigma rudimentary, anterior lobe oblique, slightly flattened. Capsule included in the persistent calyx, oblong-linear, glabrous, 2-4-seeded, separating with difficulty at maturity into 2 valves, 1-2 seeds to each valve held in position by retinacula. Seeds flattened, suborbicular, mucilaginous when wetted.

Type species: *Dyschoriste erecta* (Burm.) O. Ktze. in *Rev. Gen. Pl.* 2: 485. 1891.

KEY TO SPECIES

1. Plants glandular-pubescent 1. *D. hirsutissima*
Plants glabrous or pubescent but not glandular 2
2. Inflorescence spicate 3
Inflorescence not spicate 4
3. Anther cells pubescent; internal surface of corolla-throat pubescent.
..... 2. *D. trichanthera*
Anther cells glabrous; internal surface of corolla-throat glabrous. 3. *D. Schottiana*
4. Leaves mostly linear 5
Leaves ovate, other than linear 14
5. Corolla approximately 10 mm. long 4. *D. angusta*
Corolla 15 mm. or more long 6
6. Corolla 15-20 mm. long 7
Corolla 25-30 mm. long 11
7. Leaves 2 mm. or more wide 8
Leaves 1 mm. or less wide 5. *D. Purpusii*
8. Anther cells pubescent 6. *D. lavandulacea*
Anther cells glabrous 9
9. Stem glabrous, except for distinct pubescence at node; flowers solitary
at node 7. *D. Greenmanii*
Stem evenly pubescent; flowers usually two or more at node 10

10. Plants low-growing, ascending, 1 dm. or less high; style approximately 6 mm. long; S. Amer. sp. 8. *D. Niederleinii*
Plants strict, 3-6 dm. high; style approximately 12 mm. long; Mex. sp. 9. *D. Schiedeana*
11. Flowers characteristically solitary at the node. 10. *D. Rosei*
Flowers not solitary at the node. 12
12. Leaves mostly linear, 2 mm. or less wide. 11. *D. jaliscensis*
Leaves linear to linear-lanceolate, more than 3 mm. wide. 13
13. Stem villous-hirsute. 12. *D. angustifolia*
Stem hirsute with rigid hairs. 13. *D. linearis*
14. Leaves distinctly dentate. 14. *D. bilabiata*
Leaves not distinctly dentate. 15
15. Cinereous-pubescent throughout. 16
Not cinereous-pubescent. 17
16. Leaf margins entire, not crenulate. 15. *D. decumbens*
Leaf margins distinctly crenulate. 16. *D. crenulata*
17. Inflorescence capitate or subcapitate. 18
Inflorescence other than capitate. 21
18. Anthers emarginate, elongate at the apex. 17. *D. capitata*
Anthers without an elongation at the apex. 19
19. Corolla 35-38 mm. long. 18. *D. Pringlei*
Corolla 20-21 mm. long. 20
20. Leaves glabrous, oblanceolate. 19. *D. oaxacensis*
Leaves pubescent, obovate-elliptic. 20. *D. pinetorum*
21. Corolla 15 mm. or less in length. 22
Corolla 20 mm. or more in length. 33
22. Leaves sessile. 23
Leaves petiolate. 25
23. Plants with converging anther cells giving a sagittate appearance; stigma lobed. 21. *D. sagittata*
Plants with parallel anther cells; stigma unlobed. 24
24. Plants with glabrous leaves; S. Amer. sp. 22. *D. Serpyllum*
Plants with pubescent leaves; Mex. sp. 23. *D. Lloydii*
25. Leaves glabrous. 26
Leaves pubescent. 28
26. Leaves 6-7 cm. long. 27
Leaves 1.5-3 cm. long. 24. *D. microphylla*
27. Anthers distinctly calcarate at base; leaves lanceolate; Mexican species. 25. *D. saltuensis*
Anthers only slightly calcarate at base; leaves ovate-elliptic; S. Amer. species. 26. *D. ciliata*
28. Stems winged; leaves approximately 10 cm. long. 27. *D. quadrangularis*
Stems not winged; leaves 2-4 cm. long. 29
29. Calyx-tube glabrous, lobes ciliate with softly hirsute hairs; leaves lanceolate. 23. *D. quitensis*
Calyx tube pubescent, lobes ciliate with stiffish hairs; leaves ovate to rotund. 30
30. Anther cells diverging, giving a sagittate appearance; corolla and calyx lobes frequently reduced to 4; incomplete didynamy common. 29. *D. maranhonis*

- Anther cells parallel; corolla and calyx characters constant; complete didynamy.....31
31. Style approximately 5 mm. long.....32
- Style 10 mm. or more long.....30. *D. hirsuta*
32. Lower leaves obovate to subrotund, emarginate at the apex.....31. *D. hygrophiloides*
- Lower leaves ovate, obtuse but not emarginate at the apex.....32. *D. repens*
33. Calyx 9-10 mm. long.....33. *D. Pulegium*
- Calyx 15 mm. or more long.....34
34. Flowers crowded in glomerules.....35
- Flowers usually in pairs at the nodes.....36
35. Stem erect; corolla barely exceeding or equalling the calyx in length;
- Mex. sp.....34. *D. ovata*
- Stem geniculate-ascending; corolla 5 mm. or more longer than calyx;
- S. Amer. sp.....35. *D. amoena*
36. Leaves pubescent.....37
- Leaves glabrous.....39
37. Villous-pubescent throughout.....36. *D. xylopoda*
- Plants not villous-pubescent.....38
38. Capsule 4-seeded; N. Amer. sp.....37. *D. oblongifolia*
- Capsule 2-seeded; S. Amer. sp.....38. *D. humilis*
39. Leaves distinctly acuminate; the two bracts at each axil nearly equalling the leaf in all characters.....39. *D. paraguariensis*
- Leaves obtuse at apex; bracts foliate but not equalling the leaf in size.....40
40. Leaves sessile; corolla 25-28 mm. long; Am. bor. sp.
-37a. *D. oblongifolia* f. *glabra*
- Leaves petiolate; corolla 20 mm. long; S. Amer. sp.....40. *D. Tweediana*

1. *Dyschoriste hirsutissima* (Nees) O. Ktze. Rev. Gen. Pl. 2: 486. 1891.

Calophanes hirsutissimus Nees in DC. Prodr. 11: 109. 1847; Hemsl. in Biol. Cent.-Am. Bot. 2: 502. 1882.

Calophanes bilobatus Rose in Contr. U.S. Nat. Herb. 1: 109. 1891.

Stems branching, ascending from a stout perennial base to a height of 10-12 dm., somewhat quadrangular, more or less pubescent with the pubescence, in some cases, restricted to the edges, occasionally glandular at the apex; leaves petioled, ovate to oblong-ovate, 3-8 cm. long, 1.5-3 cm. wide, acute at both ends, margin usually entire or slightly crenulate, sometimes slightly denticulate, pubescent on both surfaces, younger leaves often densely so and glandular; inflorescence axillary, subtended by glandular-pubescent, subulate bracts; calyx 5-lobed, subulate-setaceous, extremely glandular-pubescent, approximately 11 mm.

long, lobes more than one-half the total length of the calyx; corolla subbilabiate, puberulent on the outer surface, occasionally glandular, averaging 14 mm. in length, tube about the same length as the abruptly amplified throat; stamens adnate to a little below the middle of the corolla-throat; stigma oblique; capsule 4-seeded; seeds oblique.

Distribution: slopes of the Sierra Madre of western and southern Mexico.

Specimens examined:

Southwestern Chihuahua, Aug.-Nov. 1885, *Palmer 235* (G, US); Alamos, 180 miles s.e. from Guaymas, Sonora, alt. 418 m., 26 March-8 April, 1890, *Palmer 402* (G, US); Sierra de los Alamos Mt., 6 miles due south of town of Alamos, Sonora, 14 March, 1910, *Rose, Standley & Russell 12833* (US); dry hillside, Acaponeta, Tepic, 10 April, 1910, *Rose, Standley & Russell 14298* (US); dry rocky slopes near Guadalajara, Jalisco, 11 Dec. 1888, *Pringle 2154* (G); hills near Guadalajara, Jalisco, 15 Nov. 1889, *Pringle 2939* (FM, G); Cuernavaca, Morelos, 15 Nov. 1865, *Bourgeau 1262* (G); Cuicatlan, Oaxaca, alt. 1000 m., 9 Dec. 1895, *Gonzalez 43* (G); Monte Alban, Oaxaca, 1933 m., 23 Nov. 1894, *Pringle 6053* (G, M, US); Monte Alban, Oaxaca, alt. 2833 m., 24 Nov. 1894, *L. C. Smith 323* (G); Monte Alban, near Oaxaca City, Oaxaca, alt. 1900-2000 m., 23 Nov. 1894, *L. C. Smith 729* (M, US); Monte Alban, Oaxaca, 29 Dec. 1895, *Seler 1733* (G); Valle de Oaxaca, Oaxaca, alt. 1650 m., 18 Nov. 1906, *Conzatti 1521* (FM); Tehuantepec, June, 1906, *Gandoger* (M 120892); Hacienda de Guadalupe, date lacking, *Ehrenberg 1223* (B TYPE, M fragment and photograph).

2. *Dyschoriste trichanthera*¹ n. sp.

Pl. 4.

Dyschoriste maranhonis Lindau in Bull. Herb. Boiss. II. 3: 628. 1903, as to *Fiebrig 4856*, *Hassler 5908*, *7780*, not O. Ktze.

Stems stout, branched, erect, 5-6 dm. high, glabrate, pubescent near the apex, basal portion densely covered with cystoliths, swollen at the nodes; leaves oblong-ovate to ovate, younger

¹ *Dyschoriste trichanthera* Kob., sp. nov., caulibus suffruticosis, erectis, 5-6 dm. altis, glabrescentibus, apice pubescentibus, inferiore cystolitherissimo, tumidis ad nodos; foliis oblongo-ovatis vel ovatis, 5-7 cm. longis, 2-3 cm. latis, integerrimis vel crenulatis, petiolis 10-12 mm. longis; floribus axillaribus, aliquot ad singulares nodos, fere prope apicem spicatis; bracteis parvis, foliaceis, ciliatis, pubescentibus, bracteolis acuminatis, 4-7 mm. longis; calyce 13-14 mm. longo, lobis subulatis, setaceis, 8-9

leaves pubescent, older leaves glabrate, 5-7 cm. long not including the petiole, 2-3 cm. wide, entire to crenulate, petiole 10-12 mm. long; flowers axillary, crowded at the nodes near the apex giving a spicate appearance; bracts small, foliaceous, ciliate and pubescent, bracteoles acuminate, 4-7 mm. long; calyx 13-14 mm. long, lobes subulate-setaceous, 8-9 mm. long, often recurved at the tip, ciliate, with 2 kinds of multicellular hairs, both flaccid and delicate; corolla distinctly bilabiate, 10-20 mm. long, rose or violet, lobes obtuse, emarginate, puberulent on external surface, distinctly pubescent on the internal surface; stamens barely included, adnate to about opposite the labiation of the corolla, anthers pubescent, style filiform, 10-20 mm. long, stigma oblique, linear; capsule not seen.

Distribution: along rivers, northern Paraguay.

Specimens examined:

In the region of the river Capivary, Paraguay, date lacking, Hassler 5908 (G); between the rivers Apa and Aquidaban, Paraguay, Jan. 1908-9, Fiebrig 4856 (G); in region along the river Apa, Paraguay, Nov. 1901, Hassler 7780 (G TYPE, M photograph and fragment).

3. *Dyschoriste Schottiana* (Nees) Kobuski, n. comb.

Hygrophila Schottiana Nees in Mart. Fl. Bras. 9: 22. 1847; Nees in DC. Prodr. 11: 87. 1847.

Dyschoriste crinita (Nees) O. Ktze. Rev. Gen. Pl. 2: 485. 1891; Lindau in Bull. Herb. Boiss. 7: 575. 1899.

Calophanes crinitus Nees in Mart. Fl. Bras. 9: 26. 1847; Nees in DC. Prodr. 11: 107. 1847.

Herbaceous perennial; stem erect, 5-6 dm. high, profusely branched, hirsute; leaves oblong-lanceolate, 4-5 cm. long, 1-1.5 cm. broad, tapering below into a short petiole, entire, glabrous, margin and midrib of under surface scabrous; inflorescence axillary, cymose, many-flowered, subtended by bracts; calyx deeply

mm. longis, apice saepe recurvatis, ciliatis cum duobus generibus multicellularum capillorum, ambis flaccidis subtilibusque; corolla bilabiata, 10-20 mm. longa, rosea vel violacea, lobis obtusis, emarginatis, extus puberulentis, interiore anterioris lobis faucorum pubescentibus; staminibus parce inclusis, antheris pubescentibus; stylo 10-20 mm. longo, stigmata obliqua, lineari; capsula ignota.—TYPE collected along the river Apa, Paraguay, Nov. 1901, E. Hassler 7780 (G).

5-parted, densely hirsute, about 10 mm. long, lobes subulate-setaceous; corolla more or less bilabiate, approximately 18–20 mm. long, pubescent on the external surface; capsule 8–9 mm. long, 4-seeded, glabrous; seeds flattened, suborbicular.

Distribution: southeastern Brazil.

Specimens examined:

Prov. Goyaz, Brazil, Feb. 1841–42, *Gardner 3951* (K TYPE, M photograph).

4. *Dyschoriste angusta* (Gray) Small, Fl. Miami, 168. 1913; Small, Fl. Florida Keys, 135. 1913.

Calophanes angusta Gray, Syn. Fl. N. Am. ed. 2, 2¹: 456. 1886; Small, Fl. Southeastern U.S. ed. 1, 1083. 1903, and ed. 2, 1913.

Calophanes oblongifolia var. *angusta* Gray, Syn. Fl. N. Am. ed. 1, 2¹: 324. 1878, and ed. 2, 1886.

A low-growing perennial, 1–2 dm. high; stem erect or ascending from a creeping base, slightly puberulent, occasionally branching; leaves many, subsessile, linear to linear-lanceolate, 1.5–2 cm. long, lineolate, entire; bracts foliaceous, about one-half as long as the leaves; flowers axillary; calyx 8–9 mm. long, lobes subulate-setaceous, ciliate, distinct to near the base, hardly surpassing the capsule at maturity; corolla blue, purple, or rarely, white, slightly bilabiate, approximately 1 cm. long, tube slightly shorter than the limb and a little amplified at the base; stamens adnate to the base of the limb of the corolla; anthers ovate, filaments widening at the base; capsule glabrous, linear, 4-seeded; seeds somewhat oblique.

Distribution: southern Florida.

Specimens examined:

Palm Beach County: Palm Beach, 26 Dec. 1895–11 Jan. 1896, *Hitchcock 1455* (FM).

Dade County: pine lands, Grossmanns, 24 May, 1904, *Britton 155* (FM); Cocoanut Grove, 26 Dec. 1895, 11 Jan. 1896, *Hitchcock 1456* (FM); Miami, near river, 21 Nov. 1903, *Eaton 385* (FM); in pine lands between Cocoanut Grove and Cutler, 13–23 Nov. 1903, *Small & Carter 776* (FM); cabbage field in pine woods, Grossmanns, 25 Feb. 1905, *Eaton 1247* (G); Biscayne Bay, 1874, *Palmer 347* (G, M, US); Miami, May, 1877, *Garber* (G, M

120923); Biscayne Bay, June, 1880, *Curtiss 1938* (G); Lemon City, 3 March, 1892, *Simpson 528* (G, US); rocky, calcareous land, Miami, 6 April, 1897, *Curtiss 5858* (M, FM, G, US); Black Point Bridge near Cutler, 27 Feb. 1920, *Young 301* (US).

5. *Dyschoriste Purpusii*¹ n. sp.

Pl. 5.

Perennial, erect or ascending from a suffruticose base, branching quite profusely, more or less pubescent, with short, stout, white hairs; leaves sessile, linear to linear-lanceolate, 18–20 mm. long, 1 mm. or less broad, entire, pubescent; flowers slightly pedicellate, subtended by two foliaceous bracts; calyx 5-parted, 9–11 mm. long, lobes unequal, slightly longer than the tube, pubescent, ciliate; corolla 15–17 mm. long, pubescent on the external surface, tube very narrow, not amplified at the base, approximately 7 mm. long, limb 4 mm. long; anthers of the shorter pair of stamens occasionally smaller; style 11–12 mm. long, stigma oblique; capsule linear, glabrous, approximately 10 mm. long; seeds 4, oblique.

Distribution: south Mexico.

Specimens examined:

Puebla: rocky hills, Tehuacan, June, 1905, *Purpus 2362* (M TYPE, US, G, FM); vicinity of San Luis Tultitlanapa, July, 1908, *Purpus 3347* in part (M).

6. *Dyschoriste lavandulacea* (Nees) O. Ktze. Rev. Gen. Pl. 2: 486. 1891.

Calophanes lavandulaceus Nees in Mart. Fl. Bras. 9: 27. 1847; Nees in DC. Prodr. 11: 112. 1847.

Stems erect from a perennial base, 1.5–2 dm. high, sparingly pubescent, quite angular; leaves sessile, linear-lanceolate, 40–50

¹ *Dyschoriste Purpusii* Kob., sp. nov., caulibus perennis, erectis vel ascendentibus a suffruticosi basi, ramis profusis, plus minusve pubescentibus cum brevibus albidis capillis; foliis sessilibus, linearibus vel lineari-oblongatis, 18–20 mm. longis, 1 mm. minusve latis, integerrimis, pubescentibus; floribus parum pedicellatis, subtendentibus bracteis; calyce 5-diviso, 9–11 mm. longo, lobis inaequalibus, paulo tubo longioribus, pubescentibus, ciliatis; corolla extus puberula, 15–17 mm. longa, tubo angustissimo, non ampliato ad basem, plus 7 mm. longo, limbo 4 mm. longis; staminibus postero-lateralibus minoribus; capsula lineari, glabra, 10 mm. longa, 4-sperma.—TYPE collected on rocky hills, Tehuacan, June, 1905, C. A. Purpus 2362 (M).

mm. long, 4-5 mm. wide, tapering to an acute apex, entire, glabrous; inflorescence somewhat glomerulate, several-flowered; bracts minute, 3-5 mm. long, resembling the calyx in texture; calyx 5-lobed, 13-14 mm. long, tube about 5 mm. long, glabrous and covered with cystoliths except for the ciliate, subulate-setaceous lobes; corolla 5-lobed, slightly bilabiate, 20 mm. long, pubescent on the external surface, tube one-half the total length of the corolla, lobes quite truncate; anther cells slightly puberulent; mature capsule not seen.

Distribution: south-central Brazil.

Specimens examined:

In dry fields, near Rio Pardo, Brazil, Sept. 1826, *Riedel 501* (L TYPE, M photograph).

7. *Dyschoriste Greenmanii*¹ n. sp.

Pl. 6.

Plants about 2 dm. high, ascending from a perennial base; stems slender, branched, pubescent at the nodes, otherwise quite glabrous; leaves sessile, linear, 20-25 mm. long, approximately 2 mm. broad, entire, ciliate, sparingly pubescent; flowers few, solitary at the nodes, subtended by 2-foliate bracts; calyx deeply 5-parted, 15 mm. long, flaccid-pubescent on the main nerves; lobes subulate-setaceous, approximately 10 mm. long; corolla pubescent on the external surface, 17 mm. long, scarcely exceeding the calyx in length, tube 6.5-7 mm. long, throat more or less equalling the tube in length; style 12 mm. long, pubescent, stigma oblique; capsule linear, glabrous, 7-8 mm. long, 4-seeded.

Distribution: northeastern Mexico.

Specimens examined:

Vicinity of Victoria, Tamaulipas, alt. 320 m., 1 May-13 June, 1907, *Palmer 492* (US TYPE, M photograph and fragment).

¹ *Dyschoriste Greenmanii* Kob., sp. nov., planta prope 2 dm. alta, ascendens a perenne basi; caulibus gracilibus, ramosis, pubescentibus ad nodos, aliter glabris; foliis linearibus, sessilibus, 20-25 mm. longis, 2 mm. latis, integerrimis, ciliatis, parce pubescentibus; floribus paucis, solitariis ad nodos, subtendentibus 2-foliaceis bracteis; calyce profunde 5-diviso, 15 mm. longo, flaccido-pubescente nervis, lobis subulato-setaceis, prope 10 mm. longis; corolla 17 mm. longa, paulo calyce longiore, tubo 6.5-7 mm. longo, fauce plus minusve aequante tubum; stylo 12 mm. longo, pubescente, stigmata obliqua; capsula lineari, glabra, 7-8 mm. longa, 4-sperma.—TYPE collected in the vicinity of Victoria, Tamaulipas, Mexico, 1 May-13 June, 1907, *E. Palmer 492* (US).

8. *Dyschoriste Niederleinii* Lindau in Engl. Bot. Jahrb. 19 (Beibl. 48): 15. 1894.

Low-growing perennial; stems ascending, about 1 dm. high, branches tetragonal, minutely puberulent; leaves linear, approximately 30 mm. long, 5 mm. broad, somewhat obtuse at the apex, entire, glabrous, sparsely pilose at the base, petiole 2 mm. long; flowers single, axillary, subtended by small bracts; calyx 5-parted, puberulent, 11 mm. long, tube and calyx lobes of equal length; corolla puberulent on the external surface, 15 mm. long, ventricose; style 6 mm. long, filiform, pubescent; mature capsule unknown.

Distribution: Argentina.

Specimens examined:

"Ad Primer Misionero de Hernandez," Puck and Fernandez (Niederlein 42), Argentina, Feb. 1884, (B TYPE, M photograph).

9. *Dyschoriste Schiedeana* (Nees) O. Ktze. Rev. Gen. Pl. 2: 486. 1891.

Calophanes Schideanus Nees in DC. Prodr. 11: 111. 1847; Mueller in Walpers, Ann. 5: 647. 1858, including var. *multiflorus*; Hemsl. in Biol. Cent.-Am. Bot. 2: 502. 1882.

Perennial, ascending or erect from a suffrutescent base, 3-6 dm. high; stems somewhat angular, hirsute, branched near the base; leaves usually linear-lanceolate, lower cauline leaves occasionally obovate, 20-25 mm. long, 3-5 mm. broad, acute at the apex, narrowed at the base into a very short petiole, entire, hirsute on both surfaces; flowers axillary, usually two in an axil, subtended by bracts which equal or nearly equal the calyx in length; calyx 5-parted, 11-12 mm. long, lobes 7 mm. long, subulate-setaceous, hirsute; corolla 14-15 mm. long, pubescent on external surface, tube 4 mm. long; mature capsule 7-8 mm. long, linear, glabrous, acute at apex, 4-seeded; seeds typical.

Distribution: eastern Mexico.

Specimens examined:

Nuevo Leon: near Monterey, alt. 550 m., Aug. 1911, Arséne 6411 (US).

Vera Cruz: in fields near Jalapa, date lacking, Schiede 122 (M photograph of type, B TYPE); Mirador, date lacking, Sartorius (US 55268).

10. *Dyschoriste Rosei*¹ n. sp.

Pl. 7, fig. 1.

Low-growing perennial; stem pubescent, branched, ascending or erect, 12–15 cm. high; leaves sessile, linear, entire, glabrous, 18–25 mm. long, 2 mm. broad; flowers few, solitary at the nodes, usually near the apex of the stem, slightly pedicellate, subtended by 2-foliate bracts; calyx 5-parted, glabrous except for the ciliate margin of the unequal, subulate-setaceous lobes, shorter posterior lobes 8–9 mm. long, anterior lobes 11–12 mm. long; corolla externally pubescent, 25 mm. long, tube 10 mm. long, diverging abruptly into a broadly amplified throat which is approximately equal the tube in length; stamens occasionally incompletely didynamous; ovary 2-celled, each cell containing 2 ovules, style 17–18 mm. long, stigma oblique, 2 mm. long; mature capsule not seen.

Distribution: western Mexico.

Specimens examined:

Durango: without definite locality, 13 Aug. 1897, *Rose 2259* (US TYPE, M fragment and photograph).

Jalisco: on road between Mesquite and Monte Escolebo, 26 Aug. 1897, *Rose 3581* (US).

11. *Dyschoriste jaliscensis*² n. sp.

Pl. 8.

Stems several, 3–4 dm. high, erect from a ligneous, perennial base, branching, pubescent; leaves linear to linear-oblongate, 2.5–3.5 cm. long, 2 mm. or less broad, narrowed at the base,

¹*Dyschoriste Rosei* Kob., sp. nov., humilis perennis; caule pubescente, ramis ascenduntibus vel erectis, 12–15 cm. altis; foliis linearibus, sessilibus, 18–25 mm. longis, 2 mm. latis, integerrimis, glabris; floribus paucis, solitariis ad nodos, fere prope apicem, subpedicellatis, subtendentibus bracteis; calyce 5-diviso, glabro, lobis inaequalibus, subulato-setaceis, posterioribus lobis 8–9 mm. longis, anterioribus lobis 11–12 mm. longis; corolla extus puberula, 25 mm. longa, tubo 10 mm. longo, divergente subito in late ampliato fauce; staminibus didynamis, subinde imperfectis; ovario biloculo, stylo 17–18 mm. longo; stigma obliqua, 2 mm. longa; capsula ignota. —TYPE collected in the state of Durango, 13 Aug. 1897, *Rose 2259* (US).

²*Dyschoriste jaliscensis* Kob., sp. nov., planta 3–4 dm. alta; caulibus pluribus, erectis a lignoso basi, ramis pubescentibus; foliis linearibus, linear-oblongatis, 2.5–3.5 cm. longis, 2 mm. minusve latis, basi attenuatis, integerrimis, pubescentibus; floribus maioribus, bracteis foliaceis, 10 mm. longis; calyce 17–18 mm. longo, pubescente, lobis 11–12 mm. longis, subulato-setaceis, ciliatis; corolla prope 30 mm. longa, tubo 12–13 mm. longo, pauca fauce longiore; antheris 2 mm. longis, basi bicalcaratis; stylo 20–21 mm. longo, stigmata obliqua, capsula ignota. —TYPE collected on rocky hills near Guadalajara, Jalisco, 27 June, 1893, *Pringle 5481* (G).

entire, pubescent; flowers comparatively large, subtended by 2-foliaceous bracts which are about 10 mm. in length; calyx 17–18 mm. long, pubescent, lobes 11–12 mm. long, subulate, setaceous, ciliate; corolla approximately 30 mm. long, tube 12–13 mm. long, slightly longer than the throat; anthers about 2 mm. long; style 20–21 mm. long, stigma oblique; mature capsule not seen.

Distribution: western Mexico.

Specimens examined:

Jalisco: rocky hills near Guadalajara, 27 June, 1893, *Pringle 5481* (US, G TYPE, M photograph and fragment).

12. *Dyschoriste angustifolia* (Hemsl.) O. Ktze. Rev. Gen. Pl. 2: 485. 1891.

Calophanes angustifolius Hemsl. in Biol. Cent.-Am. Bot. 2: 502. 1882.

Stem erect, strict, 4–5 dm. tall, more or less villous-hirsute; leaves subsessile, linear-lanceolate, 1.5–2.5 cm. long, 3–5 mm. broad, acute at the apex, attenuate at the base, entire, scabrous; flowers axillary, disposed in dense shortly pedunculate cymes in the axils of the upper leaves, subtended by narrow bracts which almost equal the calyx in length; calyx deeply 5-parted, 15 mm. long, scabrous, lobes subulate-setaceous, nearly equalling the tube of the corolla, ciliate; corolla bilabiate, puberulent on the external surface, approximately 25 mm. long; anther cells shortly mucronate at the base; ovary 2-celled, cells 2-ovulate, glabrous, stigma linear, oblique; mature capsule not seen.

Distribution: southern Mexico.

Specimens examined:

Oaxaca: without definite locality, coll. of 1842, *Ghesbreght* (K, M photograph).

13. *Dyschoriste linearis* (Torr. & Gray) O. Ktze. Rev. Gen. Pl. 2: 486. 1891; Lindau in Engl. & Prantl, Nat. Pflanzenfam. 4th: 302. 1895; Lindau in Bull. Herb. Boiss. II. 6: 844. 1906.

Dipteracanthus linearis Torr. & Gray, Bost. Jour. Nat. Hist. 5: 50. 1845 (Pl. Lindh. 1: 50).

Calophanes linearis Gray, Syn. Fl. N. Am. 2^d: 324. 1878, and

ed. 2, 1886; Hemsl. in Biol. Cent.-Am. Bot. 2: 503. 1882; Small, Fl. Southeastern U. S. 1083. 1903, and ed. 2, 1913.

Calophanes oblongifolius var. *texensis* Nees in DC. Prodr. 11: 108. 1847; Torr. in Emory's Rept. U.S. & Mex. Bound. Surv. 2 (Bot.): 122. 1859.

Calophanes ovatus Nees in DC. Prodr. 11: 108. 1847, not *Ruellia ovata* Cav.

Ruellia ovata Benth. Pl. Hartweg. 89. 1842, not Cav. i.e., as to plant of Drummond from Texas.

Stem 18-42 cm. high, erect and strict, branched and diffuse, hirsute with both rigid and short hairs, sometimes sparsely pubescent or nearly glabrous, not cinereous; leaves linear-oblancoolate to oblong-spathulate, 1.8-6.5 cm. long, entire, lineolate, rather rigid, pubescent on midrib and veins, margin ciliate; bracts foliaceous, frequently in short-leaved specimens equalling the length of the leaf; calyx 5-cleft, densely lineolate, giving the appearance of appressed hairs, lobes 9-13 mm. long, subulate-setaceous, more or less hispid, ciliate, calyx tube 4.5-6 mm. long, in most cases one-half the length of the lobes; corolla somewhat bilabiate, 26-27 mm. long, pubescent on external surface, tube 5-7 mm. long and slightly shorter than the abruptly amplified limb; anther cells oblong; capsule 4-seeded; seeds flat.

Distribution: Texas to New Mexico and northern Mexico.

Specimens examined:

Texas: rocky prairies, 12 July, 1903, *Reverchon* (M 120836); western Texas, 1890, *Nealley* (Ch 254803); 1846, *Lindheimer* 504 (US); *Drummond* 2 no. 178 (G TYPE); *Drummond* 256 (G); dry prairies, Bay City, Matagorda Co., 6 May, 1916, *Palmer* 9667 (M); prairies, Ganado, Jackson Co., 20 March, 1916, *Curtiss* 9216 (M); dry open ground, Vanderbilt, Jackson Co., 10 May, 1916, *Palmer* 9708 (M); Calhoun Co., 10 Aug. 1920, *Drushel* 4136 (M); dry rocky prairies near Dallas, Dallas Co., June-July, *Curtiss* 1941 (FM, M); dry rocky prairies, Dallas, Dallas Co., May-June, 1879, *Reverchon* (FM 88468); dry rocky prairies near Dallas, Dallas Co., date lacking, *Reverchon* 1941 (G, M, US); rocky limestone prairies, Dallas Co., 15 May, *Reverchon* 722 (M, US); Dallas Co., 23 May, 1903, *Bebb* 1348 (FM); rocky prairies, Dallas Co., 18 May, 1900, *Reverchon* 2114 (M); field and gardens, Fort Worth,

Tarrant Co., 8 June, 1909, *Ruth 104* (US); along roadsides near Fort Worth, Tarrant Co., 5 July, 1909, *Ruth 30* (FM); dry grounds near Fort Worth, Tarrant Co., 1 June, 1910, *Ruth 81* (FM); Austin, Travis Co., 25 June, 1920, *Tharp 733* (US); Austin, Travis Co., 1897, *Buckley* (M 120803); dry hills, Austin, Travis Co., 13 May, 1872, *Hall 431* (G); dry prairies, Austin, Travis Co., 16 May, 1872, *Hall 428* (US); along Corpus Christi Bay, Nueces Co., alt. sea level, 9-12 April, 1894, *Heller 1529* (G, M US, FM); dry open ground, Strawn, Palo Pinto Co., 27 June, 1918, *Palmer 14252a* (M); Dublin, Erath Co., 1893, *Maxwell 49* (Ch); Round Top Mt., Comanche Co., 9 May, 1900, *Eggert* (M, 120809); Gillespie Co., date lacking, *Jermy 472* (M); rich hillside, Boerne, Kendall Co., 19 May, 1916, *Palmer 9811* (M); in dried river beds of mountain rivers north of Braunfels, Comal Co., 1846, *Lindheimer 325* (M); in pastures, Bracken, Comal Co., 3 Aug. 1903, *Groth 230* (G); humid prairie and along margin of shrubs near New Braunfels, Comal Co., May, 1848, *Lindheimer 677* (G, M, FM, US); in grass and on black prairie loam, New Braunfels, Comal Co., May, 1846, *Lindheimer 111* (G, M); Comanche Springs, New Braunfels, Comal Co., May, 1851, *Lindheimer 1063* (M, G, FM, US); New Braunfels, Comal Co., May, 1851, *Lindheimer 552* (M); in open pastures, 5 miles south of San Antonio, Bexar Co., 14 May, 1920, *Schultz 146* (US); San Antonio, Bexar Co., 1918, *Slater* (US 891769); San Antonio, Bexar Co., 1884, *Havard* (Ch 252081); Bexar Co., date lacking, *Jermy 62* (M, US); San Antonio, date lacking, *Jermy 249* (G); San Antonio, Bexar Co., 27 April, 1911, *Mr. & Mrs. Clemens 1069* (M); Bexar Co., date lacking, *Jermy 31* (US); San Diego, Duval Co., 1885, *Croft 6465* (M); San Diego, Duval Co., July, 1885, *Croft 6660* (M); dry open ground, Baird, Callahan Co., 26 May, 1918, *Palmer 13698* (M); Abilene, Taylor Co., 22 May, 1902, *Tracy 8079* (G, M, FM, US); prairie north of Abilene, Taylor Co., 7 June, 1900, *Eggert* (M 120800); calcareous banks, Menard Co., 11 May, 1917, *Palmer 11871* (M); dry alluvial soil along creek, Lacey's ranch, Kerr Co., 10 June, 1917, *Palmer 12229* (M); rocky ground, Sweetwater, Nolan Co., 27 May, 1918, *Palmer 13758* (M); Knickerbocker ranch, Dove Creek, Tom Green Co., May, 1880, *Tweedy 180* (US); Fort Clark, Kinney Co., 10 May, 1893, *Mearns 1432*

(US); Devils River, Valverde Co., May, 1913, *Orcutt 6230* (M); prairie north of Stanton, Martin Co., 13 June, 1900, *Eggert* (M 120810); western Texas to El Paso, New Mexico, El Paso Co., May–Oct. 1849, *Wright 432* (G, FM, US).

New Mexico: Slaughter Canyon, Guadalupe Mts., 12–20 Aug. 1924, *Standley 40624* (US).

Mexico: Sierra Madre, 45 miles south of Saltillo on border of states of Coahuila and Nuevo Leon, July, 1880, *Palmer 2033* (G); roadside, Piedras Nigras, Coahuila, May, 1883, *Havard* (Ch 267840, US 147426); near Huasemote, Durango, 15 Aug. 1897, *Rose 3495* (US).

14. *Dyschoriste bilabiata* (Seemann) O. Ktze. Rev. Gen. Pl. 2: 486. 1891; Lindau in Bull. Herb. Boiss. 7: 575. 1899. Pl. 9. *Calophanes bilabiatus* Seem. Voy. H. M. S. Herald, 324. pl. 65. 1852–57; Hemsl. in Biol. Cent.-Am. Bot. 2: 502. 1882.

Stems 6–7 dm. high, erect from a perennial base, branching, pubescent; leaves ovate-oblong, 4–5 cm. long, 1.5–2 cm. broad, acute at the apex, narrowed at the base into a petiole, repand-denticulate, densely pubescent on both surfaces; flowers axillary, cymose, cymes pedunculate, 3–5-flowered, subtended by subulate bracts; calyx 5-lobed, 12 mm. long, tube 5 mm. long, pubescent, lobes subulate-setaceous, ciliate; corolla subbilabiate, pale blue, 14 mm. long, tube 5–6 mm. long, subventricose, pubescent on the external surface; filaments hairy; ovary glabrous, style filiform, stigma linear, oblique; mature capsule linear, glabrous, 4-seeded.

Distribution: western Mexico.

Specimens examined:

Cerro de Pinal, Sinaloa, Dec. 1848, *Seemann 1513* (K TYPE, M photograph only).

15. *Dyschoriste decumbens* (Gray) O. Ktze. Rev. Gen. Pl. 2: 486. 1891; Lindau in Engl. & Prantl, Nat. Pflanzenfam. 4^b: 302. 1895.

Calophanes decumbens Gray, Syn. Fl. N. Am. ed. 1, 2¹: 325. 1878, and ed. 2, 1886; Hemsl. in Biol. Cent.-Am. Bot. 2: 502. 1882.

Calophanes oblongifolius Torr. Bot. Mex. Bound. Surv. 122. 1855, not Don.

Cinereous-puberulent throughout; stems mostly spreading on the ground from a ligneous base, occasionally erect, branched; leaves spatulate to oblanceolate, 2–3 cm. long, 0.5–1.1 cm. broad, entire, apex usually obtuse, sometimes slightly mucronate, base attenuated, often having the appearance of a petiole; flowers few, in foliose, bracteate clusters; calyx 15–20 mm. long, at maturity exceeding the capsule by as much as 10 mm., 5-cleft, tube 5–7 mm. long, lobes subulate-setaceous, hardly twice the length of the tube; corolla purple, 18–20 mm. long, tube a little longer than the throat, slightly amplified at the base; anther cells oblong, filaments united at the base of the corolla-throat; seeds 4, sub-orbicular and flattened.

Distribution: dry soil, western Texas to Arizona, and the plateau region of northern Mexico.

Specimens examined:

Texas: Chenates region of western Texas, 1889, *Nealley 580* (357) (US); infrequent on slopes between Marfa and Alpine, 15 April, 1919, *Hanson 638* (US).

New Mexico: Valley of the Rio Grande, 1851, *Wright 1462*, 1463 (M).

Arizona: Sonoito Valley, Santa Cruz Co., alt. 1833 m., Aug. 1874, *Rothrock 637* (US); Fort Huachuca, 1890, *Patzky* (US 721394); Fort Huachuca, Cochise Co., May, 1892, *Wilcox* (US 55273, M 120796); roadway, Chiricahua Mts., Cochise Co., alt. 1400 m., 9 Oct. 1907, *Blumer 2223* (FM); Fort Huachuca, Cochise Co., 1894, *Wilcox 150* (US); foothills of Santa Rita Mts., near Greaterville, Pima Co., alt. 1666 m., 16 Sept. 1916, *Shreve 4978* (US); plains about Huachuca Mts., Aug. 1882, *Lemmon* (US 55278); locality lacking, 1875, *Rothrock* (US 55277); Fort Huachuca, Cochise Co., 26 April–21 May, 1890, *Palmer 472* (US).

Mexico: Sierra Mojado Mts., Coahuila, 19 April, 1892, *Jones 374* (US, M); near the border of Coahuila and Nuevo Leon, Feb.–Oct. 1880, *Palmer 1009* (US); Saltillo, Coahuila, alt. 1600 m., 1911, *Arsène 6472* (US); Saltillo, Coahuila, July, 1880, *Palmer 2032* (G); Saltillo, Coahuila, May, 1898, *Palmer 125* (US, M); Lerios, 15 leagues east of Saltillo near the border of Coahuila and

Nuevo Leon, alt. 3000 m., 10-13 July, 1880, *Palmer 1010 (15453)* (US, M); on road near Colatlan, Zacatecas, 31 Aug. 1897, *Rose 3615* (US); exact locality lacking, San Luis Potosi, 1897, *Schaffner 354 (647)* (US); San Luis Potosi, alt. 2000-2500 m., 1878, *Parry & Palmer 699* (FM, M, G, US); near Queretaro, 20-23 Aug. 1909, *Rose & Rose 11148* (US); San Andres Mts., Chihuahua, 22 Aug. 1900, *Trelease 352* (M); hills near Chihuahua, Chihuahua, 30 Sept. 1886, *Palmer 1075* (M); vicinity of Chihuahua, Chihuahua, alt. 1300 m., 1-21 May, 1908, *Palmer 208* (US); rocky hills near Chihuahua, Chihuahua, May, 1885, *Pringle 66* (US, G, FM); Cosihuiriachic, west of the city of Chihuahua, Chihuahua, 20 Sept. 1846, *Wislizenus 185* (M); City of Durango, Durango, 1 Aug. 1898, *Nelson 4597* (US); in the vicinity of Durango, Durango, April-Nov. 1896, *Palmer 309* (FM, US, M, G); *Palmer 930* (US, M); *Palmer 276* (US, G); Sonora, 8 Sept. 1851, *Thurber 974* (G).

16. *Dyschoriste crenulata*¹ n. sp.

Pl. 7, fig. 2.

Stems several, 1-2 dm. high, erect or ascending from a perennial, ligneous base, pubescent; leaves more or less spatulate to obovate, 2-3 cm. long, 0.6-1 cm. broad, acute to obtuse at the apex, attenuate at the base, densely cinereous pubescent, margin crenulate; calyx 5-parted, 17-18 mm. long, nearly equalling the length of the corolla, tube and lobes of nearly equal length, lobes subulate-setaceous, cinereous, ciliate; corolla 18-19 mm. long, pubescent on the external surface, throat slightly longer than the tube; anthers occasionally unequally didynamous, style 11-12 mm. long, stigma oblique; mature capsule not seen.

Distribution: south Texas, south into Tamaulipas.

Specimens examined:

¹*Dyschoriste crenulata* Kob., sp. nov., planta 1-2 dm. alta; caulibus pluribus, erectis vel ascendentibus a perenne lignoso basi, pubescentibus; foliis subsessilibus, plus minusve spatulatis vel plerumque obovatis, 2-3 cm. longis, 0.6-1 cm. latis, apice acutis vel obtusis, crenulatis, basi attenuatis, cinereo-pubescentibus; calyce 5-diviso, 17-18 mm. longo, prope aequante corollam, tubo lobes aequante, lobis subulato-setaceis, cinereis, ciliatis; corolla 18-19 mm. longa, extus puberula, fauce tubo paulo longiore; staminibus didynamis, subinde imperfectis; stylo pubescente, 11-12 mm. longo, stigmata obliqua; capsula ignota.—TYPE collected on road from "San Fernando to Jimeney," state of Tamaulipas, Mexico, 26-27 Feb., 1902, *E. W. Nelson 6804* (G).

Texas: Brazos Santiago, 1899, *Nealley 124* (357) (US).

Mexico: "San Fernando to Jimeney," Tamaulipas, 26-27 Feb. 1902, *Nelson 6604* (G TYPE, US isotype, M photograph and fragment).

17. *Dyschoriste capitata* (Oerst.) O. Ktze. Rev. Gen. Pl. 2: 486. 1891.

Calophanes capitatus Oerst. in Vidensk. Meddel. 121. 1854; Mueller in Walpers, Ann. 5: 647. 1858; Hemsl. in Biol. Cent.-Am. Bot. 2: 502. 1882.

Stems several, frequently branching and ascending from a ligneous base, 4 dm. high, subtetragonal, often geniculate, pubescence becoming more pronounced and flaccid near the apex; leaves obovate, 15-27 mm. long, 8-12 mm. broad, obtuse at the apex, attenuate into a petiole varying from a subsessile condition to 5 mm. in length, entire, ciliate, upper surface hirsute, especially on basal portion of midrib and petiole, sparingly so along midrib and veins of lower surface; flowers congested in heads at the apex of the stem and branches, subtended by oblanceolate bracts, the basal portion invested with long whitish hairs; calyx 9-10 mm. long, 5-lobed, joined for one-third its total length, possessing the same pubescence as the bracts, together giving a distinctly whitish appearance to the inflorescence, lobes subulate-setaceous; corolla 15-16 mm. long, puberulent on the external surface; filaments terminated by an emarginate, mucicous prolongation at the apex; staminodium sometimes present; ovary glabrous, stigma linear, oblique; mature capsule glabrous, 8-9 mm. long, 4-seeded.

Distribution: mountains of southern Mexico.

Specimens examined:

Prov. of San Luis Potosi, 1851, *Oersted 808*¹ (C); Sierra de San Felipe, Oaxaca, alt. 2000 m., 15 June, 1897, *Pringle 6718* (FM, G, M, US); Valley of Oaxaca, alt. 1550 m., 8 June, 1897, *Conzatti & Gonzales 282* (G).

18. *Dyschoriste Pringlei* Greenm. in Proc. Am. Acad. 40: 32. 1905.

¹ This citation refers to a photograph of the only Oersted specimen of *D. capitata* found in the Copenhagen Herbarium.

Stems several, 1-2 dm. in length, erect or ascending from a ligneous perennial base, densely hirsute-pubescent or subtomentose; leaves lance-elliptic to slightly obovate, 1.5-4 cm. long, 0.5-1.6 cm. broad, obtuse or acute, entire, narrowed below to a subpetiolate base, sparingly hirsute-pubescent on both surfaces; flowers crowded in the axils of the upper leaves, forming a subcapitate, leafy inflorescence; calyx 13-14 mm. long, densely pubescent with white flaccid hirsute hairs, divided to somewhat below the middle, divisions lance-attenuate; corolla tubular-campanulate, 3-4 cm. long, externally pubescent, more or less purplish-maculate, at least in the dried state; stamens adnate to the corolla for about one-half its length, anthers rather conspicuously calcarate; ovary glabrous, style pubescent; mature capsule not seen.

Distribution: southwestern Mexico.

Specimens examined:

Barranca of Rio Blanco near Guadalajara, Jalisco, alt. 1500 m., 22 July, 1902, *Pringle 11313* (G, FM, US); deep canyons near Guadalajara, Jalisco, 1 July, 1889, *Pringle 2907* (G TYPE, FM, M photograph).

19. *Dyschoriste oaxacensis*¹ n. sp.

Pl. 10.

Stems several, procumbent, ascending from a woody base, 1-2 dm. high, pubescent with lineolations showing through pubescence; leaves sessile, oblanceolate, occasionally somewhat spatulate, 15-20 mm. long, 3-5 mm. broad, obtuse at the apex, ciliate, sparsely pubescent or glabrous, appearing scabrous because of the irregular scattering of cystoliths; flowers axillary, congested at the apex of stem and branches, producing a capitate-like inflorescence, subtended by oblanceolate bracts, approximately 10

¹ *Dyschoriste oaxacensis* Kob., sp. nov., caulibus pluribus, procumbentibus, lineolatis, 1-2 dm. altis; foliis sessilibus, oblanceolatis, rare subspathulatis, 15-20 mm. longis, 3-5 mm. latis, ciliatis, pauce pubescentibus; floribus axillaribus, in apice caulis ramorumque capitatum congestis; bracteis plus minusve 10 mm. longis; calyce 12 mm. longo, glabro, cystolithero, lobis subulatis, setaceis, ciliatis, 7 mm. longis; corolla subbilabiata, extus puberula, 20 mm. longa, tubo 7 mm. longo; antheris basi bicalcaratis; stylo lineari, pubescente, 13-14 mm. longo, stigmata lineari, obliqua; capsula 10-11 mm. longa, glabra, 4-sperma; seminibus subrotundatis, planis, humectatis mucilaginis. —TYPE collected on calcareous hills, Las Sedas, Oaxaca, Mexico, 9 July, 1891, *Pringle 6712* (G).

mm. long, calyx about 12 mm. long, except for the lobes glabrous and covered with cystoliths, lobes subulate-setaceous, ciliate, 7 mm. long; corolla externally puberulent, 20 mm. long, tube 7 mm. long, somewhat bilabiate; stamens adnate below the middle of the corolla limb; ovary glabrous, style 13–14 mm. long, stigma linear, oblique; mature capsule 10–11 mm. long, glabrous, 4-seeded; seeds oblique, somewhat rounded, flattened.

Distribution: southern Mexico.

Specimens examined:

Oaxaca: calcareous hills, Las Sedas, alt. 2000 m., 19 July, 1891, *Pringle 6712* (M TYPE, G, FM, US); Las Sedas, alt. 2000 m., 2 June, 1907, *L. C. Smith 419* (G); Nochixtlan, alt. 2000 m., 19 June, 1907, *Conzatti 1858* (FM).

20. *Dyschoriste pinetorum*¹ n. sp.

Pl. 11.

Stems erect or ascending from a woody, perennial base, branches often arising from nodes of prostrate or erect growth of previous year, subquadrangular, 20–30 cm. high, nodes frequently 5–6 cm. distant, pubescent especially at the apex; leaves obovate-elliptic, 25–35 mm. long, 10–18 mm. broad, acute to subrotund at the apex, subsessile, attenuate at the base into a very short petiole, entire, ciliate, hirsute on both surfaces, the pubescence confined to midrib and veins on the under surface, veins conspicuous; flowers disposed in heads at the tips of the stems and branches and subtended by oblanceolate bracts; calyx 11–13 mm. long, divided two-thirds the distance to the base into five subulate-setaceous, ciliate lobes, pubescence similar to that of the bracts, together giving a canescent appearance to the leafy capitate inflorescence; corolla puberulent on the external surface,

¹ *Dyschoriste pinetorum* Kob., sp. nov., caulibus erectis vel ascendentibus a lignoso perenne basi, subquadrangularis, 20–30 cm. altis, nodis saepe 5–6 cm. diversis, pubescentibus praesertim apice; ramis saepe crescentibus ex nodis prostratorum vel erectorum caulorum antecedentis anni; foliis subsessilibus, obovato-ellipticis, 25–35 mm. longis, 10–18 mm. latis, apice acutis vel subrotundis, basi in petiolum brevissimum attenuatis, integris, ciliatis, utrinque hirsutis, praesertim ad costas nervosque subtorem; floribus apice caulis ramorumque capitatum congestis; calyce 11–13 mm. longo, diviso ad $\frac{2}{3}$ a basi in quinque subulatis, setaceis, ciliatis lobis, pubescentibus, canescentibus; corolla extus puberula, 20–21 mm. longa, tubo limbum aequante; stylo hirsuta, stigmata lineari, obliqua; capsula ignota.—TYPE collected in sandy fields under pines, near Patzcuaro, Michoacan, 31 July, 1892, *C. G. Pringle 4134* (G).

20–21 mm. long, tube and throat of approximately equal length; stamens adnate to about the middle of the corolla-tube; style hirsute, stigma linear, oblique; mature capsule not seen.

Distribution: southern Mexico.

Specimens examined:

Michoacan: sandy fields under pines near Patzcuaro, 31 July, 1892, *Pringle 4134* (G TYPE, isotypes in M, Ch, FM, US).

21. *Dyschoriste sagittata*¹ n. sp.

Pl. 12.

Low-growing perennial; stems ascending 1–2 dm. high from a ligneous base, glabrous or nearly so, densely covered with cystoliths, quadrangular, somewhat winged, branched; leaves sessile, elliptic-obovate, 15–25 mm. long, 9–12 mm. broad, usually obtuse at the apex and base, glabrous, entire; bracts slender, lanceolate, glabrous, 8 mm. long, bracteoles minute, acuminate, 2–3 mm. long; calyx 8–10 mm. long, minutely pubescent on the nerves, lobes about 5 mm. long, subulate-setaceous, sparsely and minutely ciliate; corolla pubescent on the external surface, barely exceeding the calyx in length, ventricose, slightly bilabiate, lobes rounded, margins crenate; stamens small, filaments adnate to a little below the middle of the corolla-throat, anther cells converging towards the acute apex, slightly diverging at the calcarate base, giving a sagittate appearance, approximately 0.5 mm. long; style 4–5 mm. long, minutely pubescent or glabrous, stigma lobed; mature capsule not seen.

Distribution: Paraguay.

Specimens examined:

Paraguay: in region along the Alta Parana River, 1909–10, *Fiebrig 6383* (G TYPE, M fragment and photograph).

¹ *Dyschoriste sagittata* Kob. sp. nov., humilis perennis; caulibus ascendentibus, 1–2 dm. altis a lignoso basi, glabris, cystolithis, quadrangularis, alatis, ramosis; foliis sessilibus, elliptico-obovatis, 15–25 mm. longis, 9–12 mm. latis, glabris, apice basique obtusis; bracteis angustis, lanceolatis, glabris, 8 mm. longis, bracteolis minutis, acuminatis, 2–3 mm. longis; calyce 8–10 mm. longo, puberulente in nervis loborum calycum, lobis prope 5 mm. longis, subulatis-setaceis, parce et minute ciliatis; corolla extus puberula, minor calyce paululo longiore, ventricosa, subbilabata, lobis rotundis, marginibus crenatis; antheris sagittatis, basi divergentibus et bicalcaratis, apice acutis; stylo 4–5 mm. longo, parce pubescente vel glabro, stigmata trilobata, medio lobo longissimo, circiter 0.5 mm. longo; capsula ignota.—TYPE collected in the region along the Alta Parana River, Paraguay, coll. of 1909–10, *Fiebrig 6383* (G).

22. *Dyschoriste Serpyllum* (Nees) O. Ktze. Rev. Gen. Pl. 2: 486. 1891.

Calophanes Serpyllum Nees in Mart. Fl. Bras. 9: 26. 1847; Nees in DC. Prodr. 11: 110. 1847.

Stems 1 dm. or less high, erect from a suffruticose base, pubescent; lower leaves ovate, upper leaves ovate-lanceolate, narrowed at the base, 10–12 mm. long, 3–5 mm. wide, entire, glabrous, subsessile; flowers few, usually toward the apex of the stem, subtended by leafy, glabrous bracts; calyx unequally 5-parted, submembranaceous, sparsely pubescent, 12 mm. long, lobes greatly attenuated, approximately twice as long as the tube, ciliated; corolla 12–13 mm. long, pubescent on the external surface, tube very short, not more than 3–4 mm. long, ampliation into throat apparently beginning at the base of the tube; stamens abruptly yet obtusely appended at the apex, bicalcarate at the base; ovary 2-celled, each cell possessing 2 ovules, style sparsely pubescent, filiform, stigma oblique; mature capsule not seen.

Distribution: southeastern Brazil.

Specimens examined:

Brazil: in dry fields near Rio Pardo, Sept. 1826, *Riedel 45* (L TYPE, M fragment and photograph).

23. *Dyschoriste Lloydii*¹ n. sp.

Pl. 13.

Stems branched near the base, erect, pubescent, 1–1.5 dm. high; leaves sessile, oblong-ob lanceolate, 18–20 mm. long, 3–4 mm. broad, sparingly hirsute-pubescent on both surfaces, often confined to the midrib and veins, entire; bracts foliaceous, nearly equalling the calyx in length; calyx 10–10.5 mm. long, tube 4–5 mm. long, sparingly pubescent, lobes ciliate, subulate-setaceous; corolla 14 mm. long, tube 5 mm. long, approximately equalling the throat in length; ovary and stamens typical of the genus;

¹ *Dyschoriste Lloydii* Kob. sp. nov., caulibus erectis vel ascendentibus, pubescentibus, 1–1.5 dm. altis; foliis sessilibus, oblongo-ob lanceolatis, 18–20 mm. longis, 3–4 mm. latis, integris, utrinque parce hirsuto-pubescentibus, praesertim ad costas nervosque; bracteis foliaceis; calyce prope 10–10.5 mm. longo, tubo 4–5 mm. longo, parum pubescente, lobis ciliatis, subulato-setaceis; corolla 14 mm. longa, tubo 5 mm. longo, prope aequante ampliatus faucem; capsula lineari, glabra, 7–8 mm. longa, 4-sperma; seminibus typicibus.—TYPE collected near Hacienda de Cedros, state of Zacatecas, Mexico, 1908, *F. E. Lloyd 199* (US).

capsule linear, glabrous, 7–8 mm. long, 4-seeded; seeds flattened, suborbicular, oblique.

Distribution: central Mexico.

Specimens examined:

Zacatecas: flats, Hacienda de Cedros, summer 1908, *Lloyd 199* (US TYPE).

24. *Dyschoriste microphylla* (Cav.) O. Ktze. in Rev. Gen. Pl. 2: 486. 1891. Pl. 14.

Calophanes microphyllus (Cav.) Nees in DC. Prodr. 11: 113. 1847; Hemsl. in Biol. Cent.-Am. Bot. 2: 502. 1882.

Ruellia microphylla Cav. Ic. 6: 63, pl. 586, f. 2. 1801; Spreng. Syst. 2: 821. 1825.

Dyschoriste Jasminum O. Ktze. in Rev. Gen. Pl. 2: 486. 1891.

Calophanes Jasminum-mexicanum Nees in DC. Prodr. 11: 110–111. 1847; Hemsl. in Biol. Cent.-Am. Bot. 2: 502. 1882.

Stem rising or ascending from a perennial base, pubescent; leaves distinctly ovate, obtuse-rotund at the apex, attenuate at the base into a petiole, 1.5–3 cm. long (including petiole), 0.9–1.2 cm. broad, glabrous except for slight pubescence on midrib and margin, entire; inflorescence terminal or on rather short lateral branches, subtended by foliaceous bracts; calyx 5-parted, 12–13 mm. long, somewhat pubescent, especially on the lobes, lobes subulate-setaceous, one-half the total length of the calyx, ciliate; corolla puberulent on the external surface, 13–14 mm. long, tube 8–9 mm. long, ampliating abruptly into the short throat, lobes rounded; stamens adnate below the center of the corolla throat, filaments broadening toward the base; style filiform, pubescent, stigma amplified, posterior lobe rudimentary; capsule glabrous, 4-seeded, about 8 mm. long.

Distribution: southern Mexico.

Specimens examined:

Puebla: Chalmo y San Miguel, 1789–1794, *D. Luis Née* (Ma TYPE, M 928687, photograph); vicinity of Puebla at the Rancho Losado, alt. 2194 m., 29 Aug. 1909, *Bro. Nicolas 299* (US); vicinity of Puebla, date and number lacking, *Bro. Arsène* (US 1004058); Cerro Guadalupe, vicinity of Puebla, alt. 2200 m., June, 1908, *Arsène 1933* (M, G, US); entre les haciendas Santa

Barbara et Cristo, sur l'Alseseca, alt. 2150 m., 27 June, 1907, *Arséne* 1528 (US); Santa Barbara, Puebla, 1 June, 1907, *Arséne* 1075 (US, M).

Mexico: hills in the valley of Mexico, alt. 2500 m., 24 Aug. 1902, *Pringle* 11322 (G, US).

Michoacan: vicinity of Morelia, Punguato, alt. 2100 m., 16 July, 1909, *Arséne* 3044 (M, US); *Arséne* 52a (FM, US); Morelia, alt. 2000 m., 4 Aug. 1910, *Arséne* (US 1134412); vicinity of Morelia, north of Zapote, alt. 1950 m., 4 Aug. 1910, *Arséne* 5728 (M, G, US); vicinity of Morelia, Cuincho, alt. 1900 m., 1 July, 1909, *Arséne* 7303 (M, US).

25. *Dyschoriste saltuensis* Fernald, Proc. Am. Acad. 33: 92. 1898.

A slender, erect, suffrutescent plant; stems branching, subtetragonal, densely covered with short appressed hairs, ciliate at the nodes; leaves lanceolate, obtuse at the tips, tapering below into a short petiole, the lower cauline, 6 cm. long, 1.5 cm. broad, the upper scarcely half as large, above covered with cystoliths, beneath strigilose-pubescent on the midrib; flowers axillary, solitary or in glomerules of 2 to 5, peduncles 3 or 4 mm. long; bracts minute; calyx hirsute, 8–10 mm. long, divided half way to the base into 5 lance-subulate lobes; corolla light purple, pubescent without, 15 mm. or less in length, the slender tube equalling the calyx and spreading into a campanulate throat; lobes oblong, truncate, 4 mm. long; filaments hirsute, style hirsute; mature capsule approximately 10 mm. long, glabrous; seeds 4, flat, oblique.

Distribution: mountains of southwestern Mexico.

Specimens examined:

Guerrero: vicinity of Acapulco, Oct. 1894–March 1895, *Palmer* 506 (G TYPE, M, FM, Ch, US).

26. *Dyschoriste ciliata* (Nees) O. Ktze. Rev. Gen. Pl. 2: 486. 1891; Lindau in Engl. Bot. Jahrb. 19 (Beibl. 48): 15. 1894.

Calophanes ciliatus Nees in DC. Prodr. 11: 110. 1847.

Ruellia ciliata Ruiz in DC. Prodr. 11: 110. 1847.

Stem procumbent, glabrescent, with the apex and ascending

branches puberulent; lower leaves more or less spatulate, 3 cm. long, 1 cm. broad, upper leaves ovate to elliptic, 6-7 cm. long, 2-3 cm. broad, obtuse to acute at the apex, entire, nearly glabrous, the base cuneate-decurrent into a petiole about 1.5 cm. long; flowers axillary, in glomerules, subsessile, subtended by oblong, ciliate bracts; calyx 5-parted, 11 mm. long, joined for more than one-half the total calyx-length, lobes subulate-setaceous, ciliate; corolla infundibuliform, a little longer than the calyx; anthers slightly bicalcarate at the base.

Distribution: Peru.

Specimens examined:

Peru: near Huanuco, 1787, Ruiz (B TYPE, M fragment and photograph, 927773).

27. *Dyschoriste quadrangularis* (Oerst.) O. Ktze. Rev. Gen. Pl. 2: 486. 1891; Greenm. in Proc. Am. Acad. 33: 487. 1898.

Calophanes quadrangularis Oerst. Vidensk. Meddel. 120. 1854; Mueller in Walpers, Ann. 5: 647. 1858; Hemsl. in Biol. Cent.-Am. Bot. 2: 503. 1882.

Stem erect, 8-10 dm. high, distinctly quadrangular, with ciliated wings; cystoliths especially at the swollen nodes which are quite distant; leaves ovate, oblong, 7-10 cm. long, 2-3 cm. broad, acute at the apex, attenuate at the base into a petiole, repand, crenulate; flowers verticillate, in cymose clusters at the nodes; calyx 5-parted, 11 mm. long, subtended by short, subulate bracts, tube glabrous, lineolate, equalling or a little shorter than the lobes which are subulate-setaceous, canescent-pubescent along the nerves, ciliate; corolla subbilabiate, 11 mm. long, tube slightly shorter than the limb; stamens adnate to the middle of the corolla-tube; anthers oblong with basal appendages about 0.5 mm. long, filaments accrescent at point of attachment; capsule lanceolate, 8 mm. long, 4-seeded.

Distribution: eastern Mexico.

Specimens examined:

San Luis Potosi: Los Canoas, 29 Aug. 1891, Pringle 5020 (G).

Vera Cruz: Potrero de Consoquitla, Nov. 1841, Liebmann (G COTYPE); rocky soil, Zacuapan and vicinity, Oct. 1906, Purpus 2263 (M, G, FM).

28. *Dyschoriste quitensis* (HBK.) O. Ktze. Rev. Gen. Pl. 2: 486. 1891; Lindau in Bull. Herb. Boiss. 5: 679. 1897.

Calophanes quitensis (HBK.) Nees in DC. Prodr. 11: 110. 1847.

Ruellia quitensis HBK. Nov. Gen. 2: 240. 1817; Kunth, Syn. 2: 37. 1837.

Stem procumbent or ascending from a woody base, 3–4 dm. high, branched, somewhat quadrangular, puberulent; leaves oblong, elliptic-lanceolate, narrowed acutely at both ends, 25–32 mm. long, 10–12 mm. broad, entire, puberulent, constricted below into a short petiole; flowers axillary, subtended by lanceolate bracts which about equal the calyx in length; calyx 5-parted, 7–8 mm. long, united for about one-half the total calyx-length, nearly as long as the corolla, lobes subulate-setaceous, quite hirsute; corolla 8–9 mm. long, pubescent on the external surface; stamens and pistils typical of the genus; mature capsule 7–8 mm. long, acute at the apex, 4-seeded; seeds typical.

Distribution: mountains of Andes, Ecuador.

Specimens examined:

Ecuador: near Quito, around Panecilli, alt. 3000 m., *Humboldt* (B TYPE, M photograph); in Andes of Ecuador, 1857–59, *Spruce 5989* (G).

29. *Dyschoriste maranhonis* (Nees) O. Ktze. Rev. Gen. Pl. 2: 486. 1891; Lindau in Engl. & Prantl, Nat. Pflanzenfam. 4^{ab}: 302. 1895.

Zahlbrucknera maranhonis Pohl in Mart. Fl. Bras. 9: 26. 1847; DC. Prodr. 11: 108. 1847.

Calophanes maranhonis Nees in Mart. Fl. Bras. 9: 26. 1847; Nees in DC. Prodr. 11: 108. 1847.

Ruellia viscosa Pavon in Mart. Fl. Bras. 9: 26. 1847; DC. Prodr. 11: 108. 1847.

Stem ascending or erect from a perennial base, 1–1.5 dm. high, pubescent, densely so at the apex, branched; leaves oblong-ob lanceolate, 20–25 mm. long, 6–8 mm. broad, obtuse at the apex, tapering to a short-petiolate base, crenulate-subrepand, lower leaves hirsute, densely so on veins of lower surface, upper leaves subtomentose; flowers axillary, subtended by lanceolate bracts; calyx usually 5-parted, 7–8 mm. long, united for about one-half

the total calyx-length, pubescent, lobes subulate-setaceous, ciliate with long flaccid hairs; corolla approximately 14 mm. long, tube gradually amplified into the throat, pubescent on the internal as well as the external surface, occasionally only 4-lobed; stamens occasionally incompletely didynamous, the anthers sagittate, appendaged at both ends, acutely appendaged at the base; ovary glabrous, style filiform, pubescent, stigma coiled; capsule linear, 9-10 mm. long; seeds 4.

Distribution: southeastern Brazil.

Specimens examined:

Brazil: St. Ignacio, date lacking, *Sellow* (B TYPE, M fragment and photograph).

30. *Dyschoriste hirsuta* (Oerst.) O. Ktze. Rev. Gen. Pl. 2: 486. 1891.

Calophanes hirsutus Oerst. in Kjoeb. Vidensk. Meddel. 71. 1877-78.

Robust perennial, suffruticose at the base; stems erect, 4-6 dm. high, branched, at first pubescent, glabrate; leaves ovate, 15-20 mm. long, 9-12 mm. broad, subrepand, pubescent on both surfaces, petiolate, with the petiole 2-3 mm. long; flowers axillary, subtended by two oblanceolate bracts; calyx 5-parted, approximately 10 mm. long, lobes united for one-third the total calyx-length, subulate-setaceous, pubescent on the nerves, ciliate; corolla 15-16 mm. long, pale violet, pubescent on the external surface, tube and throat approximately equal in length; stamens and ovary typical of the genus.

Distribution: southeastern Brazil.

Specimens examined:

Brazil: on fields between Serra da Piedade and Lagoa Santa, 2 May, 1864, *Warming* (C TYPE, M fragment and photograph).

31. *Dyschoriste hygrophiloides* (Nees) O. Ktze. Rev. Gen. Pl. 2: 486. 1891; Lindau in Engl. & Prantl, Nat. Pflanzenfam. 4^{sb}: 302. 1895.

Calophanes hygrophiloides Nees in Mart. Fl. Bras. 9: 26. 1847; Nees in DC. Prodr. 11: 109. 1847.

Stems geniculate, ascending from a ligneous perennial base,

3-4 dm. high, pubescent; leaves petiolate, 2-3.5 cm. long, 1-2 cm. wide, lower leaves obovate-subrotund, more or less emarginate at the apex, attenuate at the base into a rather short petiole, upper leaves elliptic-ovate, softly pubescent on both surfaces, margin somewhat sinuous; inflorescence axillary, in glomerules of 2-5 flowers; bracts linear-oblongate, setaceous, pubescent, shorter than the calyx, resembling calyx-lobes, bracteoles present; calyx 5-parted, total length approximately 13 mm., lobes 7-8 mm. long, extremely setaceous, villous-ciliate; corolla somewhat bilabiate, puberulent on the external surface, 13-14 mm. long, tube and throat of about equal length; stamens adnate below the middle of the corolla limb, anther cells ovate; style filiform, quite pubescent, 4 mm. or less in length; stigma curved, mature capsule not seen.

Distribution: southeastern Brazil.

Specimens examined:

Brazil: in grassy fields, Parana, 10 Oct. 1914, *Dusén 15640* (G, M photograph).

32. *Dyschoriste repens* (Nees) O. Ktze. Rev. Gen. Pl. 2: 486. 1891; Lindau in Engl. & Prantl, Nat. Pflanzenfam. 4^b: 302. 1895.

Calophanes repens Nees in DC. Prodr. 11: 109. 1847.

Ruellia repens Ruiz acc. to Nees in DC. Prodr. 11: 109. 1847, in synonymy.

Stem spreading, ascending, geniculate from a perennial base, densely pubescent, branching; branches rather short, ascending, densely foliate; leaves obovate (lower) to ovate (upper), 2.5-3.5 cm. long, approximately 1 cm. broad, obtuse to acute at the apex, tapering at the base to a distinct petiole, ciliate, entire, hirsute on the upper surface, pubescence of unequal hairs on the midrib of the under surface; flowers axillary, subtended by foliaceous bracts; calyx 5-parted, 10-11 mm. long, nearly equalling the corolla, lobes united for about one-half the total length of the calyx, subulate-setaceous, conspicuously ciliate; corolla 11-12 mm. long, pubescent on the external surface, tube comparatively broad, short; ovary 2-celled, style filiform, pubescent, 6 mm. long, stigma oblique; capsule not seen.

Distribution: Peru.

Specimens examined:

Peru: "near Cheuchin," date lacking, Ruiz (B TYPE, M fragment and photograph).

33. *Dyschoriste Pulegium* (Nees) O. Ktze. Rev. Gen. Pl. 2: 486. 1891.

Calophanes Pulegium Nees in Mart. Fl. Bras. 9: 25. 1847; Nees in DC. Prodr. 11: 109. 1847.

Stem erect from a suffruticose base, subvelutinous-pubescent; leaves sessile, obovate, 2-2.5 cm. long, approximately 1 cm. broad, obtuse at the apex, tapering at the base, crenulate; flowers axillary in sessile glomerules; calyx 5-parted, 9-10 mm. long, hirsute, joined one-quarter the distance from the base, lobes subulate-setaceous, ciliate; corolla about twice the total calyx length; stamens and pistils typical of the genus; mature capsule lanceolate, 4-seeded.

Distribution: southeastern Brazil.

Specimens examined:

Brazil: date and exact locality lacking, Sellow 173 (B TYPE, M photograph).

34. *Dyschoriste ovata* (Cav.) O. Ktze. Rev. Gen. Pl. 2: 486. 1891; Lindau in Engl. & Prantl, Nat. Pflanzenfam. 4^{sb}: 302. 1895; Lindau in Bull. Herb. Boiss. 5: 678. 1897.

Calophanes ovatus Benth. in DC. Prodr. 11: 108. 1847, as to Cavanilles plant (not Hartweg plant); Hemsl. in Biol. Cent.-Am. Bot. 2: 502. 1882.

Ruellia ovata Cav. Ic. 3: 28, pl. 254. 1794; Willd. Sp. Pl. 3: 363. 1801.

Erect perennial, 4-6 dm. high; stems quite quadrangular, pubescent, sometimes winged, occasionally branched; leaves ovate, obovate or occasionally elliptical, 3-4 cm. long, 1-1.7 cm. broad, obtuse at the apex, attenuated at the base into a very short petiole, quite glabrous with the exception of occasional stiff hairs on the midrib and veins, cystoliths abundant, giving the appearance of appressed hairs, margin entire and ciliated, sometimes slightly repand-crenulate; flowers crowded among the foliaceous bracts at the nodes, giving a glomerulate appearance; calyx 5-parted, quite glabrous, covered with cystoliths, 14-15

mm. long, joined for about one-third the total calyx-length, lobes subulate-setaceous, ciliate; corolla 20–25 mm. long, tube less than 10 mm. long, puberulent on the external surface; stamens, pistil, and fruit typical of the genus.

Distribution: southern Mexico.

Specimens examined:

Vera Cruz: Nogales, Mt. Orizaba, alt. 1400 m., 16 Aug. 1891, *Seaton 392* (Ch, G); Borrego, near Mt. Orizaba, 26 Aug. 1866, *Bourgeau 2903* (US, G).

Morelos: near Cuernavaca, 30 July, 1906, *Pringle 13838* (US); near Cuernavaca, alt. 1500 m., 28 July, 1896, *Pringle 7249* (US).

Michoacan: Morelia, alt. 2100 m., 8 Aug. 1912, *Arsène 9027* (US).

35. *Dyschoriste amoena* (Nees) O. Ktze. Rev. Gen. Pl. 2: 485. 1891; Lindau in Engl. & Prantl, Nat. Pflanzenfam. 4th: 302. 1895.

Calophanes amoenus Nees in Mart. Fl. Bras. 9: 27. 1847; Nees in DC. Prodr. 11: 110. 1847.

Stem ascending from a perennial base, approximately 3 dm. high, geniculate, branched, puberulent toward the apex, otherwise glabrous; leaves narrowly oblong-ovate to slightly oblong-ob lanceolate, 4–5 cm. long, 1–1.5 cm. broad, obtuse to acute at the apex, entire, glabrous, attenuate at the base into a very short petiole; inflorescence axillary, bracteate, glomerulate toward the apex; calyx deeply 5-parted, 2 cm. long, quite robust, pubescent, lobes less attenuate than in the majority of species, ciliate; corolla 5 mm. or more longer than the calyx, pubescent on the external surface; stamens and ovary typical of the genus; mature capsule not seen.

Distribution: southeastern Brazil.

Specimens examined:

Brazil: date and exact locality lacking, *Sellow* (B TYPE, M photograph and fragment).

36. *Dyschoriste xylopoda*¹ n. sp.

Pl. 15.

¹ *Dyschoriste xylopoda* Kob., sp. nov., caulibus erectis vel ascendentibus, basi crasse lignosae, 2–3 dm. altis, villosopubescentibus; foliis sessilibus, lanceolato-oblongis, inferioribus rare ovatis, 2.5–3.5 cm. longis, 1 cm. minusve latis, integerrimis;

Stems strict, rising from a thick woody base to a height of 2-3 dm., villous-pubescent throughout; leaves sessile or nearly so, lanceolate-oblong, or the lowermost occasionally ovate, 2.5-3.5 cm. long, approximately 1 cm. or less broad, entire; flowers 2-3 on a short peduncle in the axils of the leaves, subtended by linear-lanceolate bracts which are a little shorter than the calyx; calyx deeply 5-cleft, 17-18 mm. long, lobes 11-12 mm. long, subulate-setaceous, villous-ciliate; corolla 25-27 mm. long, pubescent on the external surface, tube 10 mm. long; stamens adnate below the middle of the corolla limb, anthers oblong-ovate, 2-3 mm. long; ovary 2-celled, glabrous, style filiform, 20 mm. long, pubescent, stigma linear, oblique, nearly 2 mm. long; mature fruit not seen.

Distribution: southern Mexico.

Specimens examined:

Jalisco: hills near Guadalajara, 19 July, 1893, *Pringle 4442* (M TYPE, G, FM).

37. *Dyschoriste oblongifolia* (Michx.) O. Ktze. Rev. Gen. Pl. 2: 486. 1891; Lindau in Engl. & Prantl, Nat. Pflanzenfam. 4^{3b}: 302. 1895. Pl. 3.

Ruellia oblongifolia Michx. Fl. Bor.-Am. 2: 23. 1805; Pursh, Fl. Am. Sept. 2: 420. 1814.

? *Ruellia biflora* L. Sp. Pl. 2: 635. 1753 (a doubtful synonym—refer to D. Don in Sweet's Brit. Fl. Garden).

Calophanes oblongifolia (Michx.) D. Don in Sweet, Brit. Fl. Gard. 2: pl. 181. 1833; Gray, Syn. Fl. N. Am. ed 1, 2¹: 324. 1878, and ed. 2, 1886; Chapman, Fl. Southeastern U.S. ed. 3, 365. 1897; Britt. & Brown, Ill. Fl. 3: 202. 1898; Small, Fl. Southeastern U.S. ed. 1, 1083. 1903, and ed. 2, 1913.

Dipteracanthus biflorus Nees in Linnaea 16: 294. 1842.

Dipteracanthus oblongifolius Chapman, Fl. Southeastern U.S. ed. 2, 303. 1889.

floribus 2-3, pedicellatis, axillaribus; bracteis linearo-lanceolatis, calyce haud paulo brevior; calyce profunde 5-diviso, 17-18 mm. longo, lobis 11-12 mm. longis; subulato-setaceis, villosociliatis; corolla 25-27 mm. longa, extus puberulenta, tubo 10 mm. longo; filamentis basi connatis, antheris ovato-oblongis, 2-3 mm. longis; stylo 20 mm. longo, pubescente, stigmata lineari, obliqua, prope 2 cm. longa; capsula ignota. —TYPE collected on hills, near Guadalajara, Jalisco, Mexico, 19 July 1893, C. G. *Pringle 4442* (M).

Herbaceous perennial; stem quadrangular, branched at the base, erect, pubescent or softly hirsute, 4-8 dm. high; leaves sessile or short-petiolate, oblong-ovate, 2.5-4.5 cm. long, 0.5-1.5 cm. broad, rounded or obtuse at the apex, narrowed at the base, entire or slightly crenulate, softly hirsute; flowers solitary, axillary on very short pedicels, subtended by narrowly oblong, leafy bracts; calyx 15-18 mm. long, deeply 5-parted, subulate-setaceous, lobes ciliate; corolla blue, usually purple-maculate in the throat, approximately 25-27 mm. long, the tube shorter than the abruptly amplified throat, puberulent on the external surface, lobes rounded; filaments slightly pubescent at point of adnation; anther cells oblong; mature capsule 10-12 mm. long, lanceolate; seeds 4, flattened, oblique.

Distribution: sandy pine barrens, southern Virginia to Florida.

Specimens examined:

Virginia: date and locality lacking, probably southeastern portion of the state, *Thurber* (G).

South Carolina: sandy ground, north of Graniteville, Aiken Co., 21 May, 1899, *Eggert* (M); locality lacking, May, 1867, *Ravenel* (G, US); barrens near Beaufort, Beaufort Co., 26 April, 1917, *Churchill* 743 (M).

Georgia: sand hills between Grovetown and Forrest, Columbia Co., 10 June, 1902, *Harper* 1312 (M, G, US); low places north of Belair, Richmond Co., 22 May, 1899, *Eggert* (M); Ocmulgee River swamp below Macon, Laurens Co., *Small* (FM); Savannah, Chatham Co., 1842, *Curtis* (M); Darien Junction, McIntosh Co., 31 May, 1909, *H. H. Smith* 2219 (FM); Darien Junction, McIntosh Co., alt. sea level, 25-27 June, 1895, *Small* (FM); Lexington, Oglethorpe Co., 1836, *Short* (M).

Florida: sandy pine lands, date and exact locality lacking, *Mohr* (US 721388); eastern Florida, 1895, *Curtiss* (US); pine barrens, Duval Co., 21 April, 1902, *Fredholm* 5101 (G, US); pine barrens, Duval Co., 28 April, 1902, *Fredholm* 5127 (G); near Jacksonville, Duval Co., 2 May, 1893, *Curtiss* 4428 (M, US); dry pine barrens, near Jacksonville, Duval Co., 8 May, 1894, *Curtiss* 4667 (FM, US); Jacksonville, Duval Co., April, 1869, *Canby* (M, G, US); dry pine barrens, near Jacksonville, Duval Co., *Curtiss* 1938 (M, G, US); south Jacksonville, Duval Co., 7 April,

1897, *Churchill* (G); dry sandy pine barrens, St. Augustine, St. Johns Co., May–Aug. 1875, *Reynolds* (M); sandy pine barrens, DeLand, Volusia Co., date lacking, *Harkness* (M); dry pine woods, DeLand, Volusia Co., 7 May, 1910, *Hood* (M); vicinity of Eustis, Lake Co., June–July, 1894, *Hitchcock* 1454 (FM, M); Eustis, Lake Co., 26 April, 1896, *Webber* 520 (M); sandy soil, high pine lands, vicinity of Eustis, Lake Co., *Nash* 184 (M, G, US); Eustis, Lake Co., 28 May–15 June, 1895, *Nash* 1774 (US); Winter Park, Orange Co., March, 1900, *Huger* 19 (M); Clarcona, Orange Co., 18–22 Aug. 1899, *Pieters* 121 (US); dry sand, Okeechobee region, Brevard Co., 2 June, 1903, *Fredholm* 5870 (G); Lake City, Jefferson Co., June–July, 1898, *Hitchcock* 1452, 1453 (FM); Rosewood, Levy Co., June, 1876, *Garber* (FM); dry sandy ground, Polk Co., 12 April, 1894, *Ohlinger* 1415 (FM); dry land, Polk Co., 11 June, 1894, *Ohlinger* 188, 1437 (FM); Lake Alfred, Polk Co., 11 June, 1922, *Armstrong & Armstrong* (M); Polk Co., March, 1890, *Milligan* (US); pine barrens, Tampa, Hillsborough Co., Aug. 1898, *Ferguson* (M); in pine lands near St. Petersburg, Pinellas Co., 10 Nov. 1907, *Deam* 2832 (G); pine woods, Manatee Co., 16 March, 1887, *Rothrock* (FM 160054, 322461); sandy field, Bradentown, Manatee Co., 15 May, 1900, *Tracy* 6683 (M); Fort Myers, Lee Co., 1904, *Westgate* 3607 (FM); Fort Myers, Lee Co., July–Aug. 1900, *Hitchcock* (FM); Aspalaga, Liberty Co., May, 1898, *Chapman* (M).

Alabama: date and locality lacking, *Buckley* (M, US).

37a. *Dyschoriste oblongifolia* (Michx.) O. Ktze. forma *glabra* n. f.

Stem and leaves glabrous; otherwise as the species.

Distribution: Florida.

Specimens examined:

Florida: Tocoí, St. Johns Co., 1874, *Palmer* 346 (M, G); Lake City, Columbia Co., 4 May, 1893, *Rolf* 190 (M, FM); Gainesville, Alachua Co., 5 June, 1910, *Hood* (M); Fort Myers, Lee Co., July–Aug. 1900, *Hitchcock* (M); cypress swamp and low pine land, vicinity of Fort Myers, Lee Co., 8 May, 1916, *J. P. Standley* 179 (M, G, FM, US); in pine land, Mullock Creek District, about 8 miles southeast of Fort Myers, Lee Co., May–June, 1917, *J. P. Standley* 443 (M, G, FM, US); in pine land, vicinity of Fort

Myers, Lee Co., 12 May, 1916, *J. P. Standley 13* (M, G, FM, US); pine woods, vicinity of Fort Myers, Lee Co., 28 Feb. 1916, *J. P. Standley 12852* (US); vicinity of Fort Myers, Lee Co., 29 Feb. 1916, *J. P. Standley 12917* (US); moist pine lands, vicinity of Fort Myers, Lee Co., 14 Dec. 1919, *P. C. Standley 18894* (US); high pine land, Jessamine, 17-20 April, 1899, *Barnhart 2680* (FM).

38. *Dyschoriste humilis* (Griseb.) Lindau in Engl. Bot. Jahrb. 19(Beibl. 48): 15. 1894; Lindau in Bull. Herb. Boiss. II. 3: 628. 1903.

Ruellia geminiflora Kth. var. *humilis* Griseb. in Pl. Lor. 176. 1874, and Symb. Argent. 259. 1879.

Stem slender, branched below, ascending from a ligneous, perennial base, pubescent; leaves oblong-elliptic, 2-3.5 cm. long, 0.4-0.5 cm. broad, tapering acutely both at the apex and at the short-petiolate base, puberulent, ciliate, entire or sinuous; flowers in twos or threes, axillary, subtended by foliaceous bracts about 8-10 mm. long; calyx in anthesis approximately 14 mm. long, puberulent, lobes setaceous, 8 mm. long, at maturity the calyx sometimes attains a length of 20 mm.; corolla 21-22 mm. long, puberulent on the external surface, tube shorter than the broadly amplified (8-9 mm. in diameter) throat; stamens adnate below the middle of the corolla throat, anther cells oblong, a little over 2 mm. long, cells of individual anthers often differing, one base distinctly acute and minutely apiculated, the other base blunt or slightly mucronate, apex of cells slightly acute; style sparsely pubescent, filiform, 19 mm. long, stigma about 2 mm. long, posterior lobe rudimentary; mature capsule exceeding the calyx lobes, 10 mm. long; retinaculum in center of each cell; seeds two, flat, oblique.

Distribution: Argentina.

Specimens examined:

Argentina: Chaco Santafichna, Mocovi, 5 Nov. 1903, *Venturi 55* (US); Cordoba, Dec. 1891, *Kuntze* (US 701502); Cordoba, 21 Dec. 1876, *Hieronymus* (FM 51116, US 282198); near the city of Cordoba, 1874-75, *Hieronymus* (FM 51115a, US 282197).

39. *Dyschoriste paraguariensis*¹ n. sp.

Pl. 16.

Stems erect, 2-3 dm. high, strict, somewhat quadrangular, nearly glabrous, sparsely pubescent at the nodes; leaves sessile, lanceolate-elliptic, 2-2.5 cm. long, 0.5-0.8 cm. wide, acute at the apex, glabrous, covered with an irregular scattering of cystoliths, margins entire, not ciliated; flowers in twos, axillary, subtended by two foliaceous bracts which resemble the leaves in nearly every respect; calyx glabrous, except for the ciliation on the lobes, covered with a regular array of cystoliths, 14 mm. long at maturity, lobes lanceolate, setaceous, 10 mm. long; corolla approximately 18 mm. long, puberulent on the external surface, tube about 9 mm. long; style filiform, about 12 mm. long, pubescent, stigma oblique; mature capsule linear, 11-12 mm. long, glabrous, 4-seeded; seeds flattened.

Distribution: Paraguay.

Specimens examined:

Paraguay: in region of the river "Tapiraguay," Aug., Hassler 4355 (G, TYPE).

40. *Dyschoriste Tweediana* (Nees) O. Ktze. Rev. Gen. Pl. 2: 486. 1891.

Calophanes Tweedianus Nees in DC. Prodr. 11: 108. 1847.

Stem ascending from a perennial base, 4-6 dm. high, pubescent; leaves ovate-elliptic, 3-3.5 cm. long, 0.9-1.5 cm. broad, acute to obtuse at the apex, tapering at the base into a very short petiole, repand-subcrenate, glabrous; flowers axillary, 1-3 aggregated on very short peduncles in the axils, subtended by oblong-lanceolate bracts which are shorter than the calyx; calyx deeply 5-parted, pubescent, 14 mm. long, lobes subulate-setaceous, ciliate; corolla infundibuliform, 5-lobed, 20 mm. long, tube 8 mm. long, pubescent on the external surface, lobes ovate, obtuse; anthers

¹ *Dyschoriste paraguariensis* Kob. sp. nov., caulibus erectis, 2-3 dm. altis, strictis plus minusve quadrangularis, subglabris, parum pubescentibus ad nodos; foliis sessilibus, lanceolato-ellipticis, 2-2.5 cm. longis, 0.5-0.8 cm. latis, apice acutis, glabris, lineolatis; floribus duobus in axillaribus, bracteis duobus, foliaceis; calyce glabro, cystolithero, 14 mm. longo, lobis lanceolatis, setaceis, 10 mm. longis; corolla violacea, 18 mm. longa, extus puberula, tubo 9 mm. longo; filamentis basi connatis; stylo 12 mm. longo, piloso; capsula lineari, 11-12 mm. longa, glabra, 4-sperma; seminibus planis.—TYPE collected in the region of the river "Tapiraguay," Paraguay, Aug., Hassler 4355 (G).

appendaged at the base, appendages connivent (according to Nees); seeds suborbicular, flattened.

Distribution: southeastern Brazil.

Specimens examined:

Brazil: in dry mountain forests in Prov. Bonar, at river Jacuhy in Rio Grande do Sul, date lacking, *Tweedie* 771 (K TYPE, M photograph).

EXCLUDED SPECIES

Calophanes californica Rose acc. to Vasey & Rose in Contr. U.S. Nat. Herb. 1: 85. 1890 = *Ruellia californica* (Rose) Johnston in Proc. Cal. Acad. Sci. IV, 12: 1171. 1924.

Calophanes cubensis A. Rich. in Sagra, Hist. de Cuba 11: 160. 1850 = *Hygrophila brasiliensis* (Spreng.) Lindau in Urb. Symb. Ant. 2: 183. 1900.

Calophanes Palmeri Gray acc. to Watson in Proc. Am. Acad. 22: 443. 1887 = *Spigelia scabrella* Benth. Pl. Hartweg. 45. 1840.

Calophanes peninsularis Rose acc. to Vasey & Rose in Contr. U. S. Nat. Herb. 1: 75. 1890 = *Ruellia peninsularis* (Rose) Johnston in Proc. Cal. Acad. Sci. IV, 12: 1172. 1924.

Dyschoriste candida Brandegee in Zoe 5: 242. 1908 = *Ruellia candida* (Brandegee) Kobuski, n. comb.

Dyschoriste cubensis Urb. Symb. Ant. 7: 381. 1912 = *Apasalus cubensis* (Urb.) Kobuski in Ann. Mo. Bot. Gard. 15: 2. 1928.

Dyschoriste diffusa Urb. Symb. Ant. 7: 380. 1912 = *Apasalus diffusus* (Urb.) Kobuski in Ann. Mo. Bot. Gard. 15: 1. 1928.

Dyschoriste humistrata (Shuttl.) O. Ktze. Rev. Gen. Pl. 2: 486. 1891 = *Apasalus humistratus* (Shuttl.) Kobuski in Ann. Mo. Bot. Gard. 15: 3. 1928.

LIST OF EXSICCATAE

The distribution numbers are printed in *italics*. Unnumbered collections are indicated by a dash. The number in parentheses is the species number used in this monograph.

Armstrong, Dr. & Mrs. G. M. — (37).

Arsène, Bro. 6411 (9); 6472 (15);

35, 52a, 1075, 1523, 1933, 3044, 5728,

7903 (24); 9027 (34).

Barnhart, J. H. 2680 (37a).

Bebb, R. 1348 (13).

Blumer, J. C. 2223 (15).

Bourgeau, M. 1262 (1); 2903 (34).

Britton, N. L. 155 (4).

Buckley, S. B. — (13); — (37).

Canby, W. M. — (37).

Chapman, G. W. — (37).

- Churchill, J. R. —, 743 (37).
 Clemens, Mr. & Mrs. J. 1069 (13).
 Conzatti, C. 1521 (1); 1858 (19).
 Conzatti, C. & Gonzalez, V. 282 (17).
 Croft, Miss M. B. 6465, 6660 (13).
 Curtis, M. — (37).
 Curtiss, A. H. 5858 (4); 1941, 9216 (13);
 —, 1938, 4428, 4667 (37).
 Deam, Mrs. C. C. 2832 (37).
 Drummond, T. 178, 256 (13).
 Drushel, J. A. 4136 (13).
 Dugès, A. — (15).
 Dusén, P. 15640 (31).
 Eaton, A. A. 385, 1247 (4).
 Eggert, H. —, — (13); — (37).
 Ehrenberg, K. 1223 (1).
 Ferguson, A. M. — (37).
 Fiebrig, K. 4856 (2); 6383 (21).
 Fredholm, A. 5101, 5127, 5870 (37).
 Gandoger, M. — (1).
 Garber, A. P. — (4); — (37).
 Gardner, J. 3951 (3).
 Ghiesbreght, A. — (12).
 Gonzalez, V. 43 (1).
 Groth, H. A. 230 (13).
 Hall, E. 428, 431 (13).
 Hanson, H. C. 638 (15).
 Harkness, W. E. — (37).
 Harper, R. M. 1312 (37).
 Hassler, E. 5908, 7780 (2); 4355 (39).
 Havard, V. — (13).
 Heller, A. A. 1529 (13).
 Hieronymus, G. — (38).
 Hitchcock, A. S. 1455, 1456 (4); —,
 1452, 1453, 1454 (37); — (37a).
 Hood, S. C. — (37); — (37a).
 Huger, A. M. 19 (37).
 Humboldt, A. — (28).
 Jermy, G. 31, 62, 249, 472 (13).
 Jones, M. E. 374 (15).
 Kuntze, O. — (38).
 Lemmon, J. G. — (15).
 Liebmann, F. M. — (27).
 Lindheimer, F. 111, 325, 504, 552, 677,
 1063 (13).
 Lloyd, F. E. 199 (23).
 Maxwell, C. F. 49 (13).
 Mearns, E. A. 1432 (13).
 Milligan, Mrs. J. M. — (37).
 Mohr, C. — (37).
 Nash, G. V. 184, 1774 (37).
 Nealley, G. C. — (13); 580 (15); 124
 (357) (16).
 Née, D. L. — (24).
 Nelson, E. W. 4597 (15); 6604 (16).
 Nicolas, Bro. 299 (24).
 Niederlein, G. 42 (8).
 Oersted, A. S. 808 (17).
 Ohlinger, L. B. 188, 1415, 1457 (37).
 Orcutt, C. R. 6230 (13).
 Palmer, Ed. 235, 402 (1); 347 (4); 492
 (7); 2033, 9667 (13); 125, 208, 276,
 309, 472, 930, 1009, 1010, 1075, 2032
 (15); 506 (25); 346 (37a).
 Palmer, E. J. —, 9708, 9811, 11871,
 12229, 13698, 13758, 14252a (13).
 Parry, C. C. & Palmer, Ed. 699 (15).
 Patzky — (15).
 Pieters, A. J. 121 (37).
 Pringle, C. G. 2154, 2939, 6053 (1);
 5481 (11); 66 (15); 6718 (17); 2907,
 11313 (18); 6712 (19); 4134 (20);
 11322 (24); 5020 (27); 7249, 13838
 (34); 4442 (36).
 Purpus, C. A. 2362, 3347 (5); 2263 (27).
 Ravenel, H. W. — (37).
 Reverchon, J. —, 722, 1941, 2114 (13).
 Reynolds, Miss M. C. — (37).
 Riedel, L. 501 (6); 45 (22).
 Rolf, P. H. 190 (37a).
 Rose, J. N. 2259, 3581 (10); 3495 (13);
 3615 (15).
 Rose, J. N. & Rose, J. R. 11148 (15).
 Rose, J. N., Standley, P. C. & Russell,
 P. G. 12833, 14298 (1).
 Rothrock, J. T. —, 637 (15); — (37).
 Ruiz, L. — (26); — (32).
 Ruth, A. 30, 81, 104 (13).
 Sartorius, C. — (9).
 Schaffner, J. G. 354 (647) (15).
 Schiede, C. J. W. 122 (9).
 Schulz, Miss E. D. 146 (13).
 Seaton, H. E. 392 (34).
 Seemann, B. 1513 (14).
 Seler, E. 1733 (1).
 Sello (Sellow), F. — (29); —, 173 (33);
 — (35).
 Short, C. W. — (37).

- Shreve, F. 4978 (15).
 Simpson, J. H. 528 (4).
 Slater, Mrs. H. D. — (13).
 Small, J. K. — (37).
 Small, J. K. & Carter, J. J. 776 (4).
 Smith, H. H. 2219 (37).
 Smith, Rev. L. C. 323, 729 (1); 419 (19).
 Spruce, R. 5989 (28).
 Standley, Miss J. P. 13, 179, 443, 12852, 12917 (37a).
 Standley, P. C. 40624 (13); 18894 (37a).
 Sharp, B. C. 733 (13).
 Thurber, G. 974 (15); — (37).
 Tracy, S. M. 8079 (13); 6683 (37).
 Trelease, W. 352 (15).
 Tweedie, J. 771 (40).
 Tweedy, F. 180 (13).
 Venturi, S. 55 (38).
 Warming, E. — (30).
 Webber, H. J. 520 (37).
 Westgate, J. M. 3807 (37).
 Wilcox, T. E. —, 150 (15).
 Wislizenus, A. 185 (15).
 Wright, C. 432 (13); 1462, 1463 (15).
 Young, J. P. 301 (4).

INDEX TO SPECIES

New genera, species, combinations and forms are printed in **bold face** type; synonyms in *italics*, and previously published names in ordinary type.

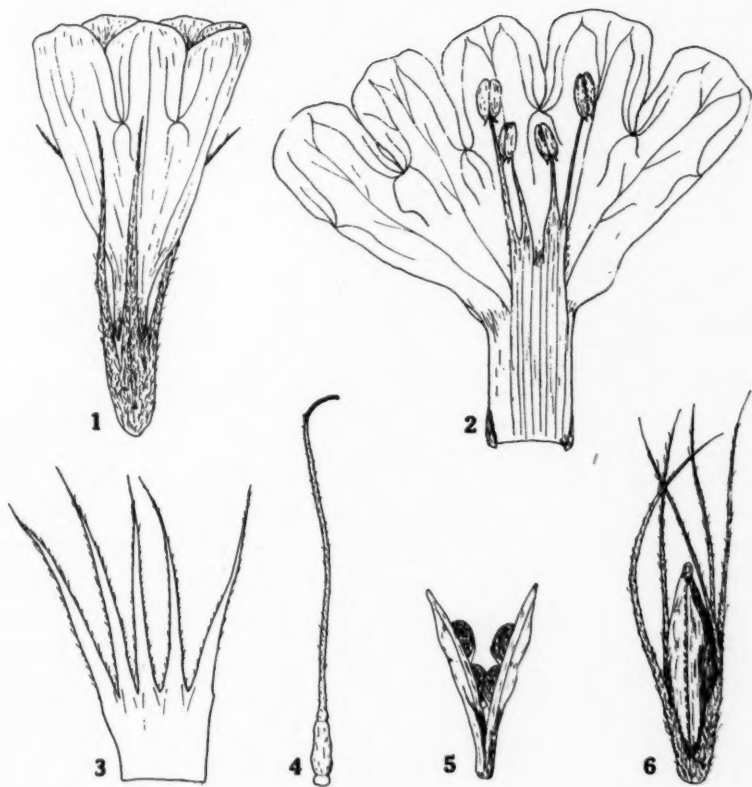
<i>Apasalus</i>	21	<i>peninsularis</i>	60
<i>cubensis</i>	60	<i>Pulegium</i>	53
<i>diffusus</i>	60	<i>quadrangularis</i>	49
<i>humistratus</i>	60	<i>quitensis</i>	50
<i>Calophanes</i>	25	<i>repens</i>	52
<i>amoenus</i>	54	<i>Schiedeanus</i>	34
<i>angustifolius</i>	36	<i>Schiedeanus</i> var. <i>multiflorus</i>	34
<i>angusta</i>	31	<i>Serpyllum</i>	46
<i>bilabiatus</i>	39	<i>Tweedianus</i>	59
<i>bulbatus</i>	28	<i>Dipteracanthus</i>	55
<i>californica</i>	60	<i>biflorus</i>	55
<i>capitatus</i>	42	<i>linearis</i>	36
<i>ciliatus</i>	48	<i>oblongifolius</i>	55
<i>crinitus</i>	30	<i>Dyschoriste</i>	25
<i>cubensis</i> A. Rich.	60	<i>amoena</i>	54
<i>decumbens</i>	39	<i>angusta</i>	31
<i>hirsutissimus</i>	28	<i>angustifolia</i>	36
<i>hirsutus</i>	51	<i>bilabiata</i>	39
<i>hygrophiloides</i>	51	<i>candida</i>	60
<i>Jasminum-mexicanum</i>	47	<i>capitata</i>	42
<i>lavandulaceus</i>	32	<i>ciliata</i>	48
<i>linearis</i>	36	<i>crenulata</i>	41
<i>maranhonis</i>	50	<i>crinita</i>	30
<i>microphyllus</i>	47	<i>cubensis</i>	60
<i>oblongifolia</i> D. Don.	55	<i>decumbens</i>	39
<i>oblongifolia</i> var. <i>angusta</i>	31	<i>depressa</i>	12
<i>oblongifolia</i> var. <i>tezensis</i>	37	<i>diffusa</i>	60
<i>oblongifolius</i> Torr.	40	<i>erecta</i>	12, 26
<i>ovatus</i> Benth.	53	Greenmanii	33
<i>ovatus</i> (Cav.) Nees	37	<i>hirsuta</i>	51
<i>Palmeri</i>	60	<i>hirsutissima</i>	28

<i>humilis</i>	58	<i>Schottiana</i>	30
<i>humistrata</i>	60	<i>Serpyllum</i>	46
<i>hygrophiloides</i>	51	<i>trichanthera</i>	29
<i>jaliscensis</i>	35	<i>Tweediana</i>	59
<i>Jasminum</i>	47	<i>xylopoda</i>	54
<i>lavandulacea</i>	32	<i>Hygrophila</i>	60
<i>linearis</i>	36	<i>brasiliensis</i>	60
<i>Lloydii</i>	46	<i>Schottiana</i>	30
<i>maranhonis</i> Lindau	30	<i>Linostylis</i>	26
<i>maranhonis</i> Nees	50	<i>Ruellia</i>	10
<i>microphylla</i>	47	<i>biflora</i>	55
<i>Niederleinii</i>	34	<i>californica</i>	60
<i>oaxacensis</i>	43	<i>candida</i>	60
<i>oblongifolia</i>	55	<i>ciliata</i>	48
<i>oblongifolia</i> f. <i>glabra</i>	57	<i>depressa</i>	10
<i>ovata</i>	53	<i>geminiflora</i> var. <i>humilis</i>	58
<i>paraguariensis</i>	59	<i>microphylla</i>	47
<i>pinetorum</i>	44	<i>oblongifolia</i>	10, 55
<i>Pringlei</i>	42	<i>ovata</i> Benth.	37
<i>Pulegium</i>	53	<i>ovata</i> Cav.	53
<i>Purpusil</i>	32	<i>peninsularis</i>	60
<i>quadrangularis</i>	49	<i>quitensis</i>	50
<i>quitensis</i>	50	<i>repens</i>	52
<i>repens</i>	52	<i>viscosa</i>	50
<i>Rosel</i>	35	<i>Spigelia</i>	60
<i>sagittata</i>	45	<i>scabrella</i>	60
<i>saltuensis</i>	48	<i>Zahlbrucknera</i>	50
<i>Schiedeana</i>	34	<i>maranhonis</i>	50

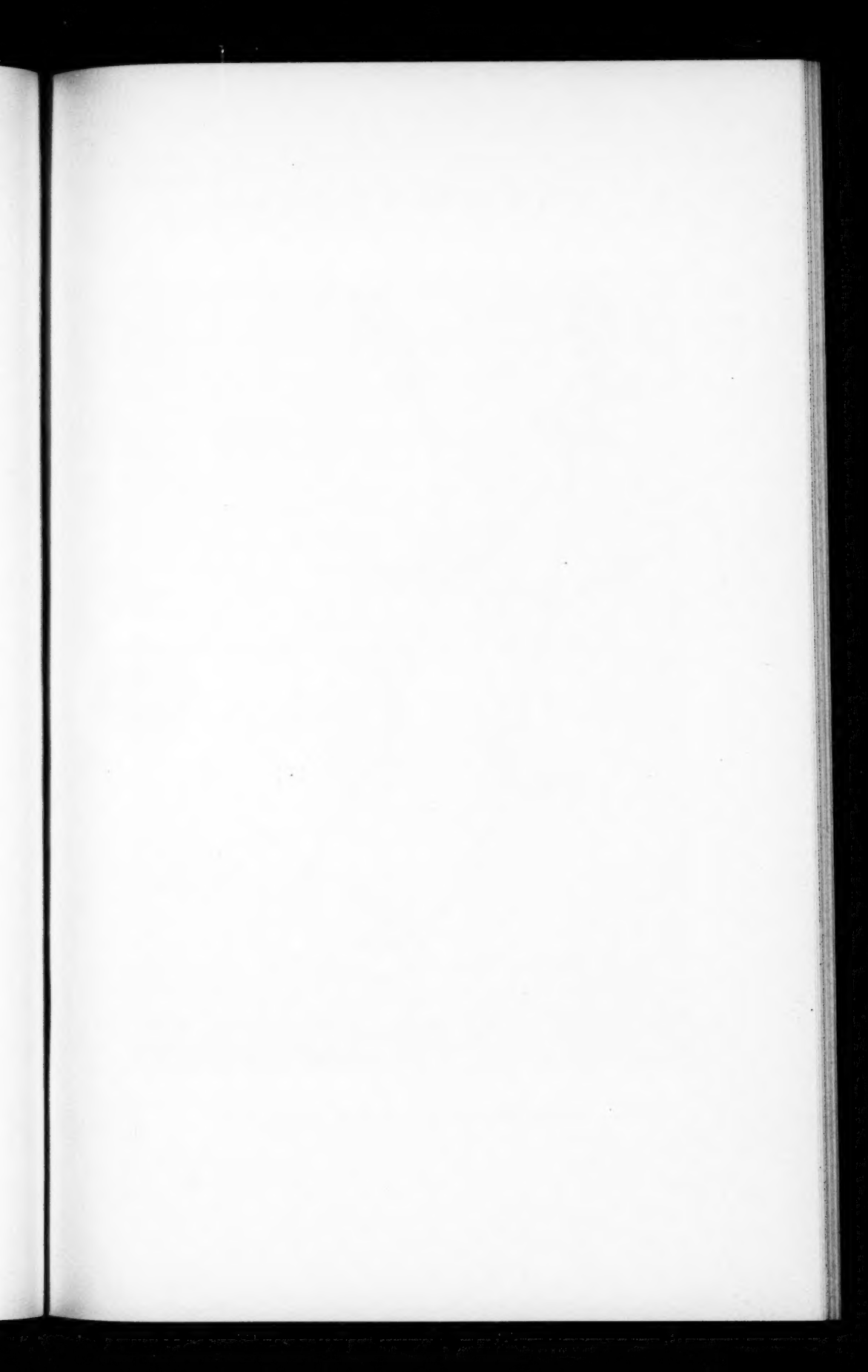
EXPLANATION OF PLATE

PLATE 3

- Fig. 1. Flower of *D. oblongifolia* (Michx.) O. Ktze. $\times 3$.
Fig. 2. Open corolla of *D. oblongifolia* (Michx.) O. Ktze. $\times 3$. Showing character and position of stamens.
Fig. 3. Open calyx of *D. oblongifolia* (Michx.) O. Ktze. $\times 3$.
Fig. 4. Pistil of *D. oblongifolia* (Michx.) O. Ktze. $\times 3$.
Fig. 5. Dehiscing capsule of *D. oblongifolia* (Michx.) O. Ktze. $\times 3$. Showing position of retinacula and seeds.
Fig. 6. Mature capsule (before dehiscence) of *D. oblongifolia* (Michx.) O. Ktze. $\times 3$. Showing the persistent calyx.



KOBUSKI—MONOGRAPH OF DYSCHORISTE



EXPLANATION OF PLATE

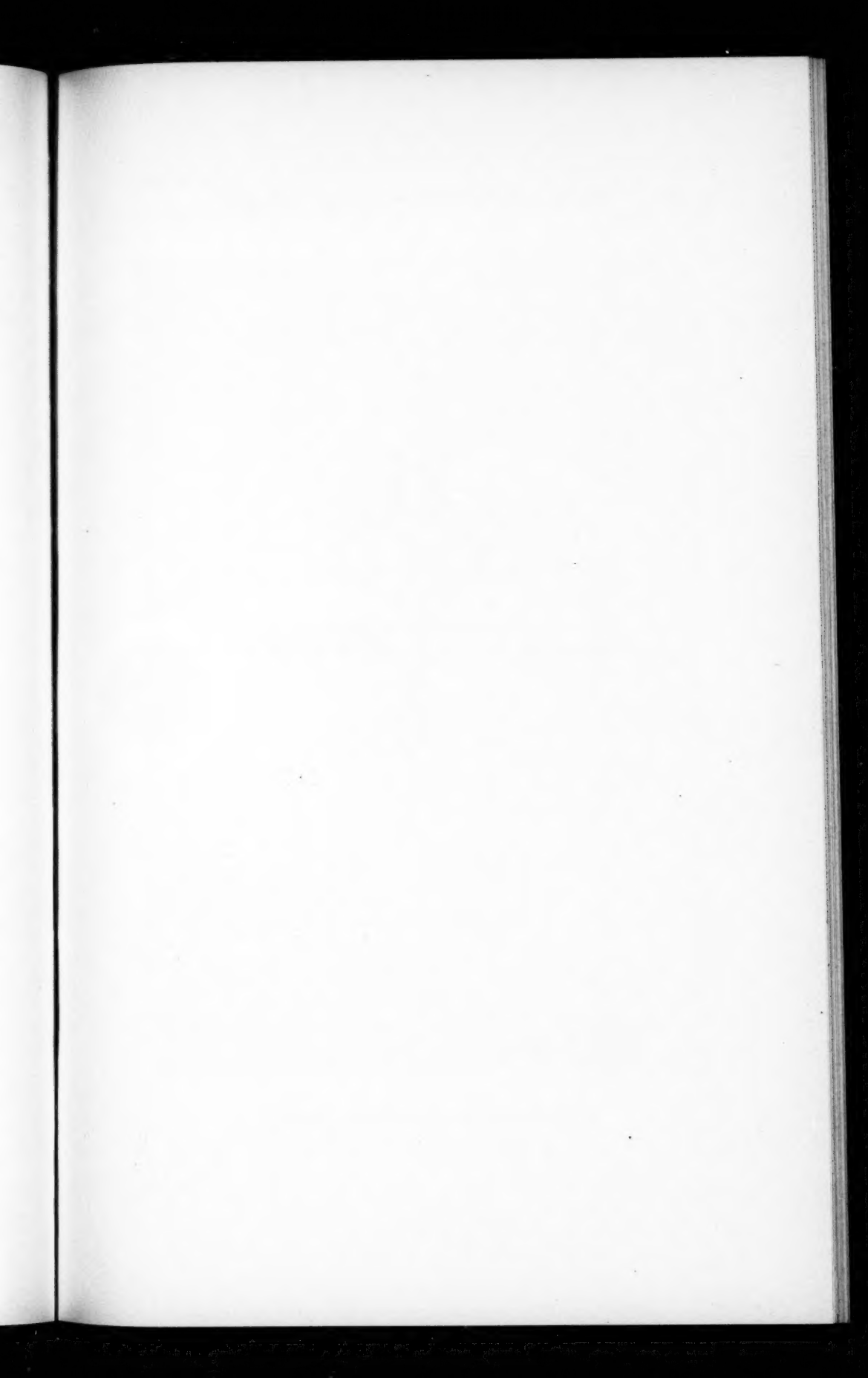
PLATE 4

Dyschoriste trichanthera Kobuski

From the type specimen, Hassler 7780, in the Gray Herbarium of Harvard University.



KOBUSKI—MONOGRAPH OF DYSCHORISTE



EXPLANATION OF PLATE

PLATE 5

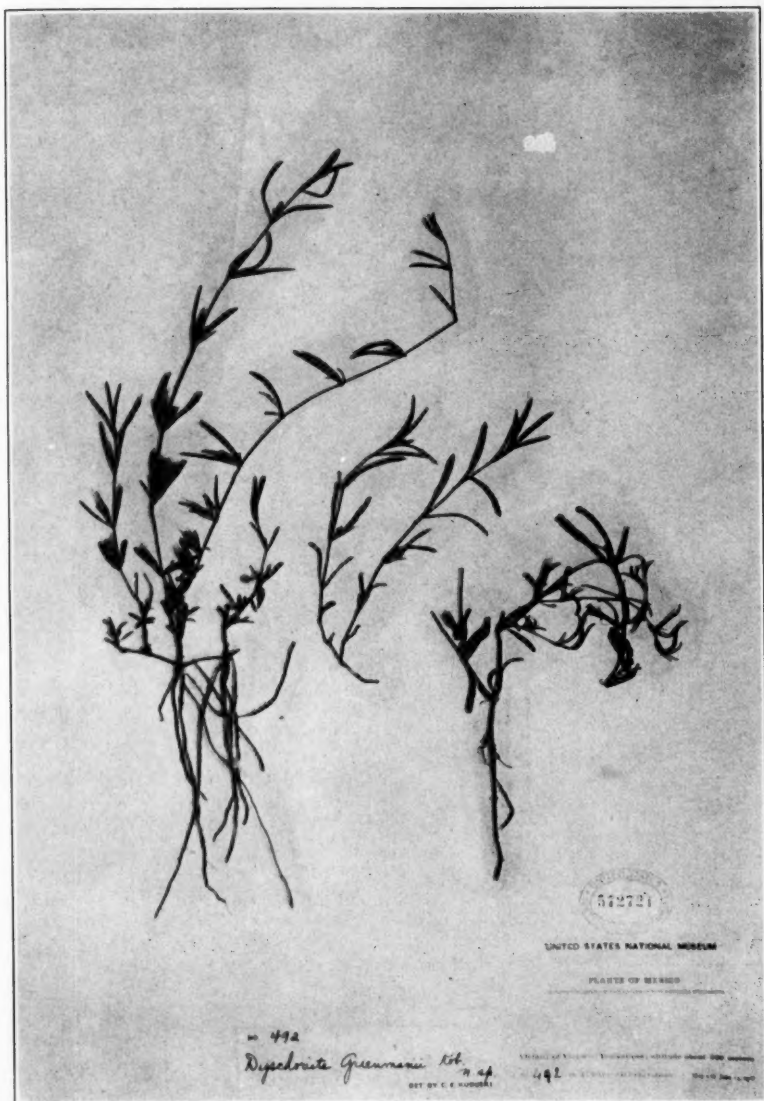
Dyschoriste Purpusii Kobuski

From the type specimen, *Purpus* 2362, in the herbarium of the Missouri Botanical Garden.

EXPLANATION OF PLATE

PLATE 6

Dyschoriste Greenmanii KobuskiFrom the type specimen, *Palmer 492*, in the United States National Herbarium



KOBUSKI—MONOGRAPH OF DYSCHORISTE

EXPLANATION OF PLATE

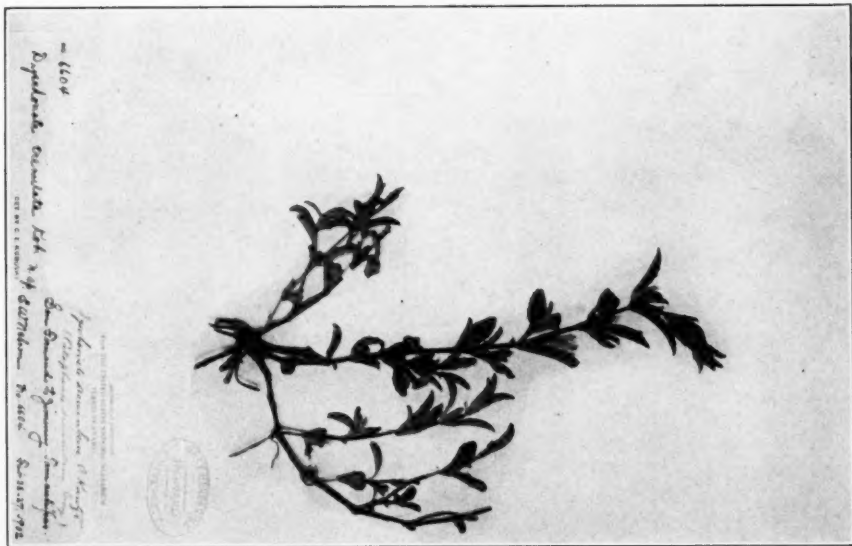
PLATE 7

Fig. 1. *Dyschoriste Rosei* Kobuski

From the type specimen, *Rose 2259*, in the United States National Herbarium

Fig. 2. *Dyschoriste crenulata* Kobuski

From the type specimen, *Nelson 6804*, in the Gray Herbarium of Harvard University.

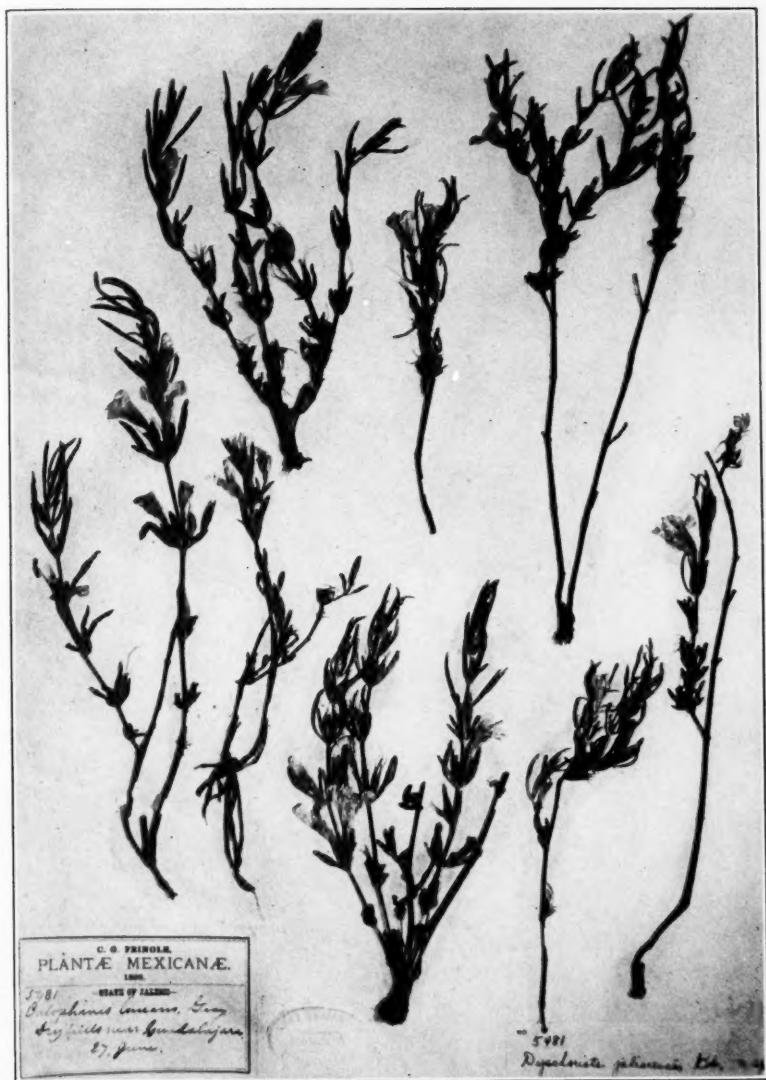


EXPLANATION OF PLATE

PLATE 8

Dyschoriste jaliscensis Kobuski

From the type specimen, *Pringle 5481*, in the Gray Herbarium of Harvard University.



KOBUSKI—MONOGRAPH OF DYSCHORISTE

EXPLANATION OF PLATE

PLATE 9

Dyschoriste bilabiata (Seem.) O. Ktze.

From the type specimen, *Seeman 1513*, in the Royal Botanic Gardens at Kew, England.



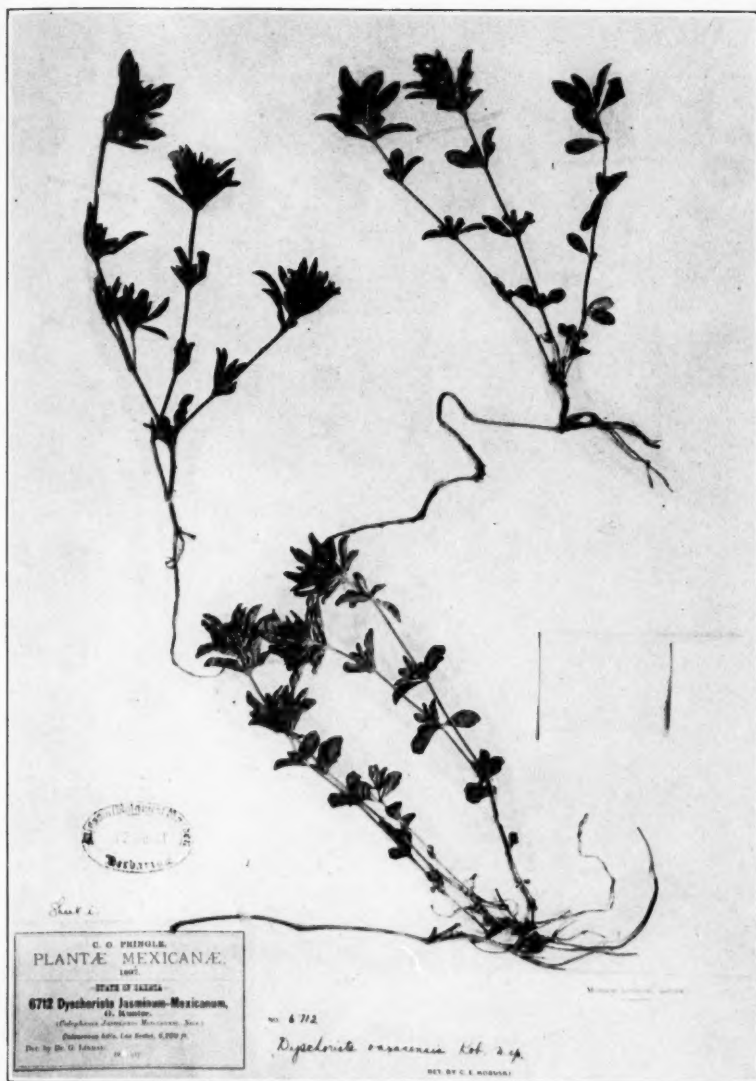
KOBUSKI—MONOGRAPH OF DYSCHORISTE

EXPLANATION OF PLATE

PLATE 10

Dyschoriste oaxacensis Kobuski

From the type specimen, *Pringle 6712*, in the herbarium of the Missouri Botanical Garden.



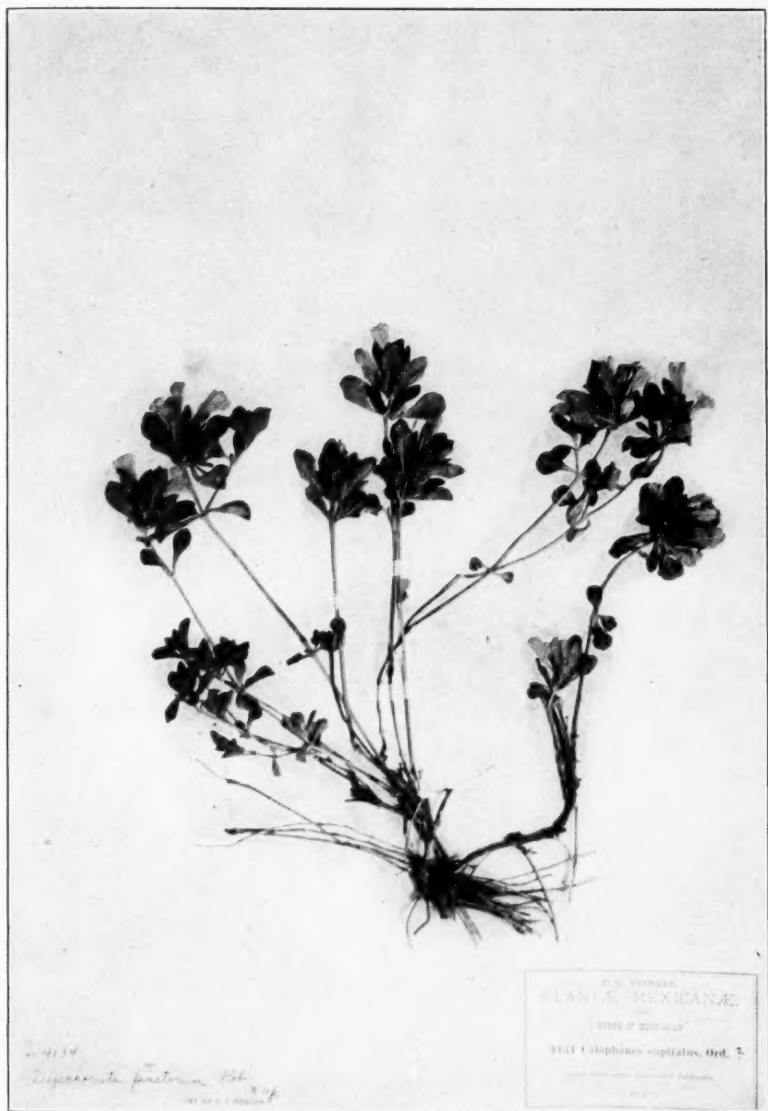
KOBUSKI—MONOGRAPH OF DYSCHORISTE

EXPLANATION OF PLATE

PLATE 11

Dyschoriste pinetorum Kobuski

From the type specimen, *Pringle 4134*, in the Gray Herbarium of Harvard University.



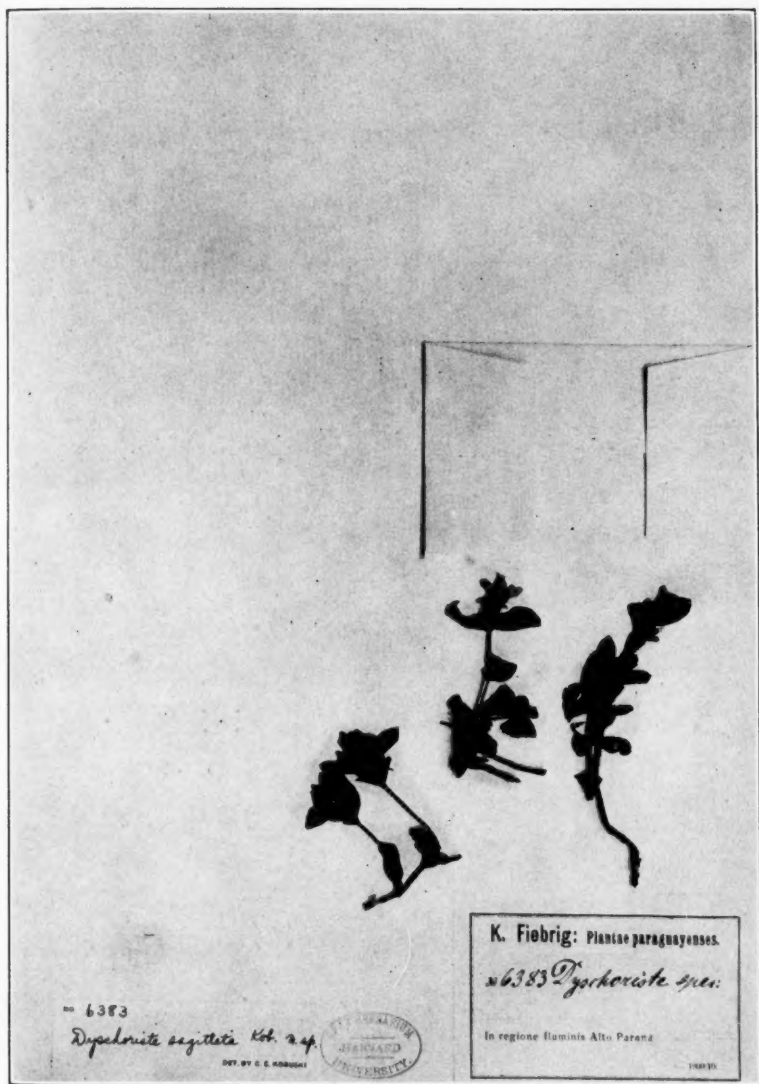
KOBUSKI—MONOGRAPH OF DYSCHORISTE

EXPLANATION OF PLATE

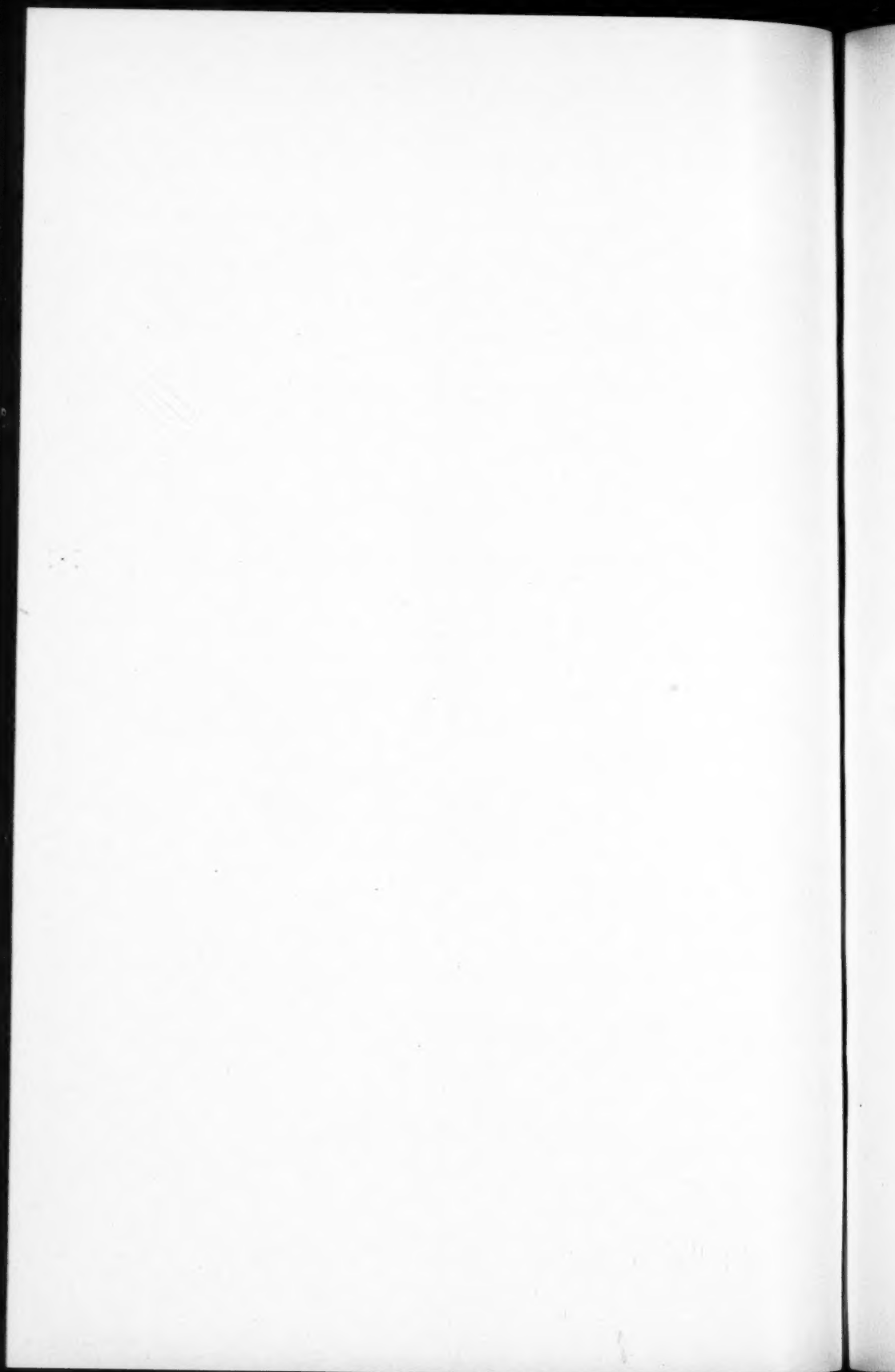
PLATE 12

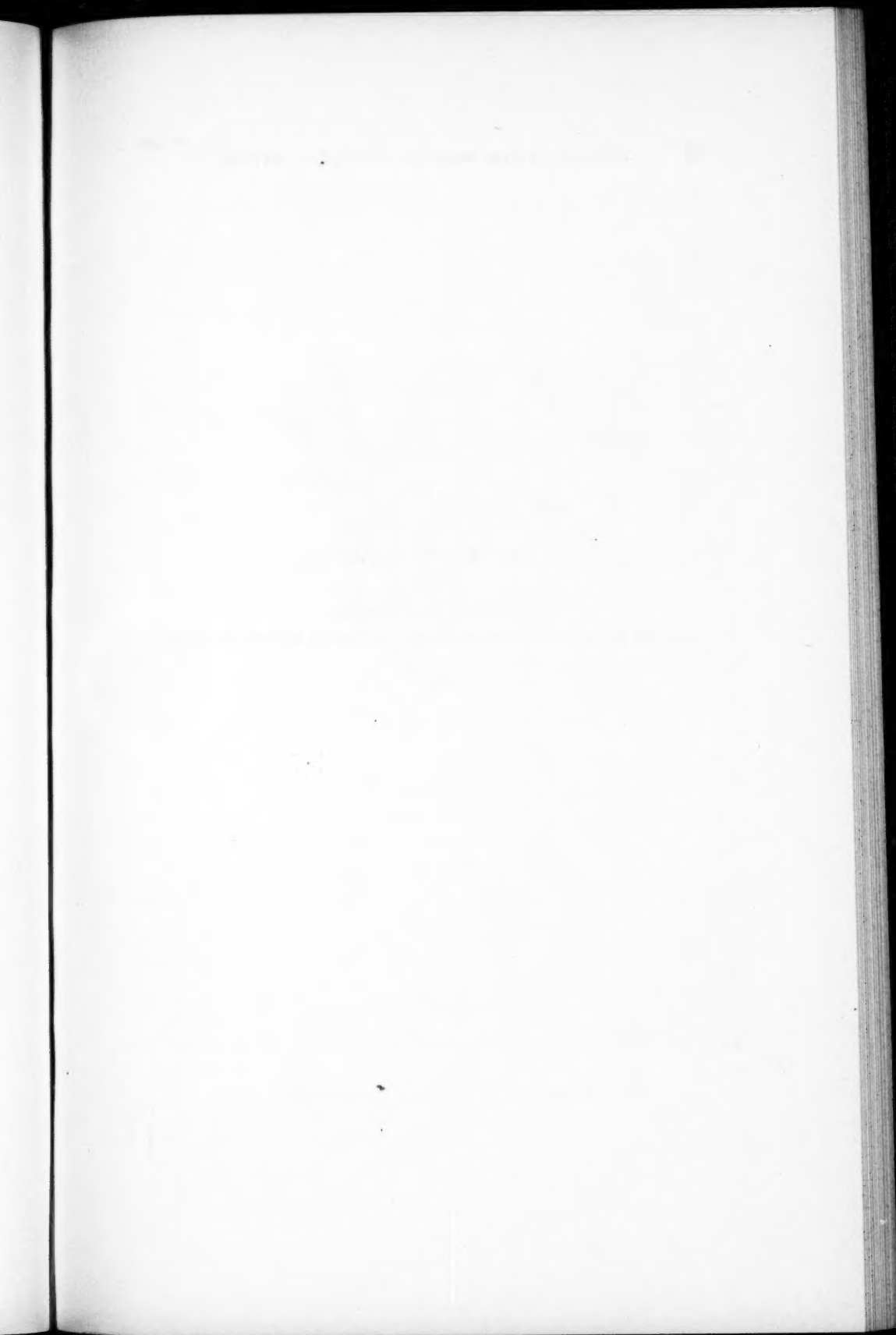
Dyschoriste sagittata Kobuski

From the type specimen, *Fiebrig 6383*, in the Gray Herbarium of Harvard University.



KOBUSKI—MONOGRAPH OF DYSCHORISTE





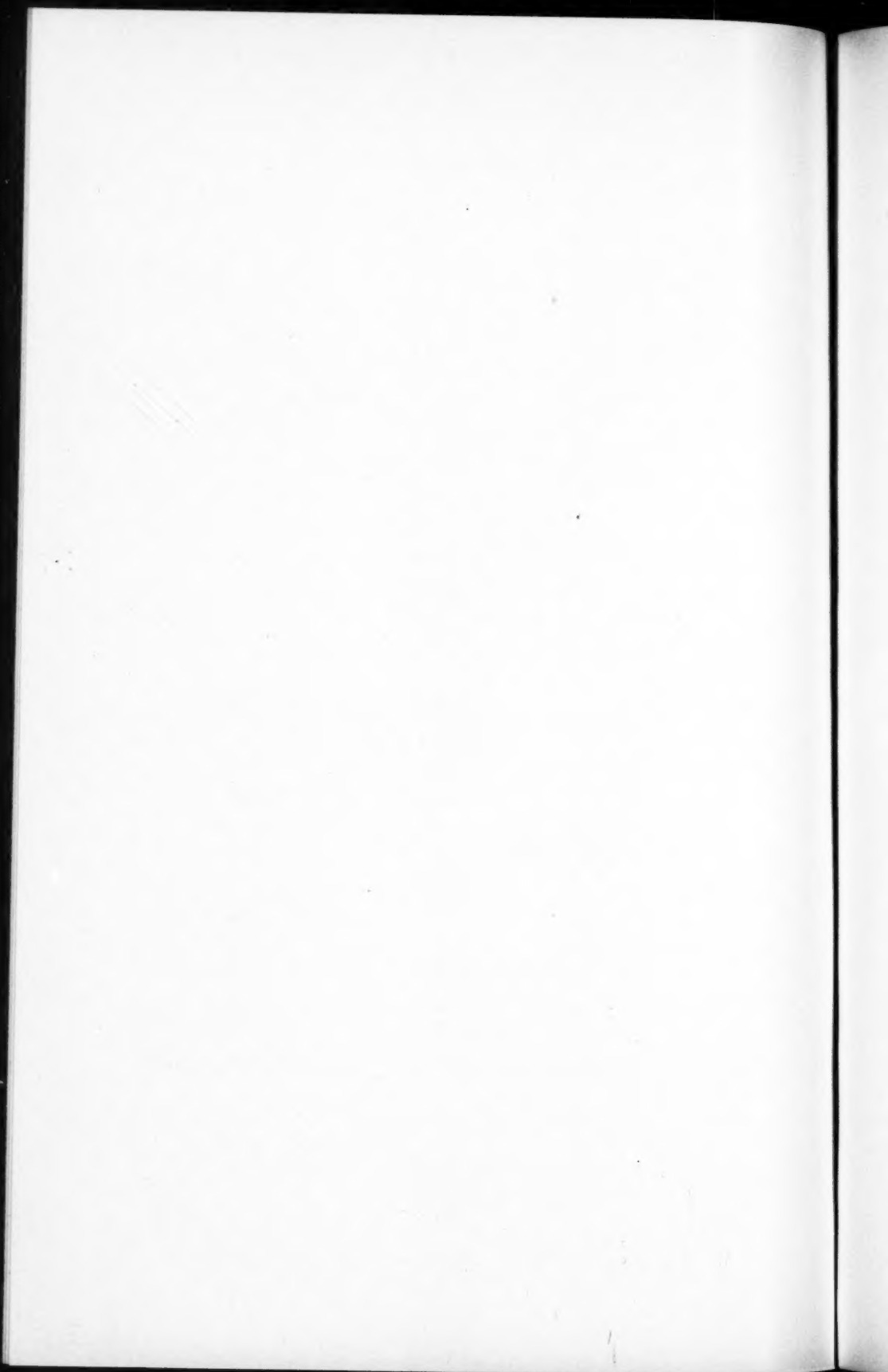
EXPLANATION OF PLATE

PLATE 13

Dyschoriste Lloydii KobuskiFrom the type specimen, *Lloyd 199*, in the United States National Herbarium.



KOBUSKI—MONOGRAPH OF DYSCHORISTE



EXPLANATION OF PLATE

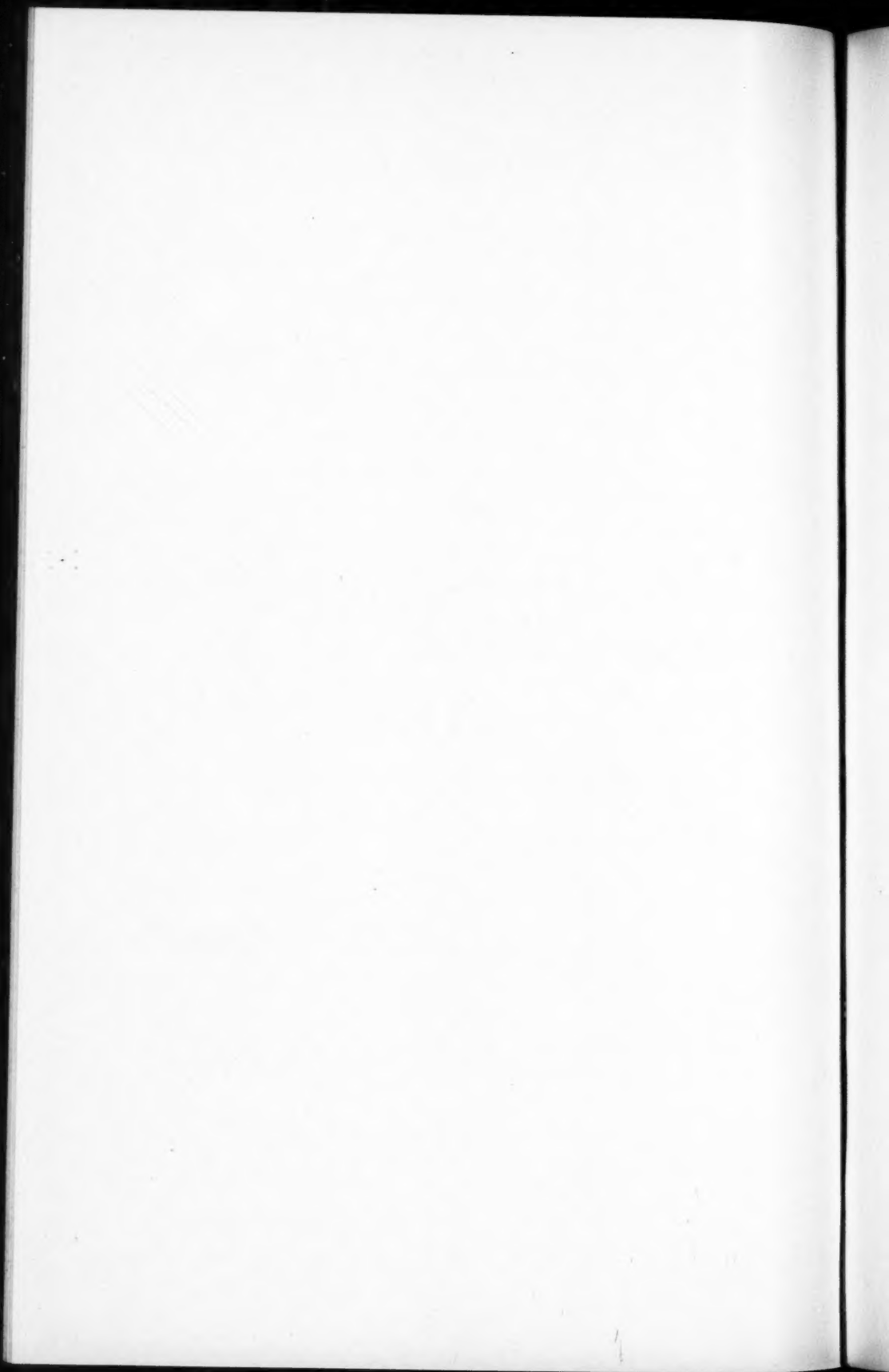
PLATE 14

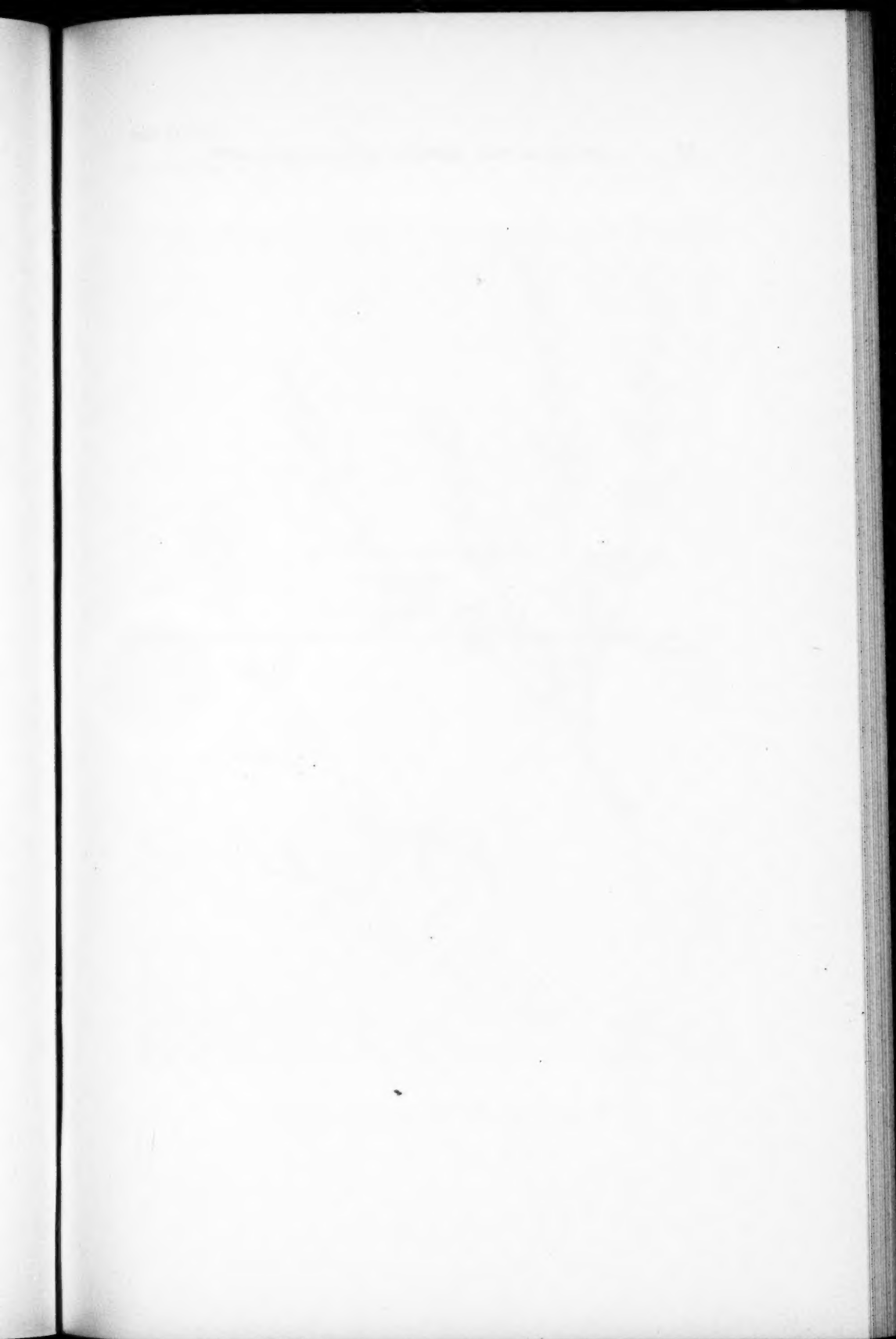
Dyschoriste microphylla (Cav.) O. Ktze.

From the type specimen, *D. Luis Née*, in the herbarium of the Botanical Garden, Madrid, Spain.



KOBUSKI—MONOGRAPH OF DYSCHORISTE





EXPLANATION OF PLATE

PLATE 15

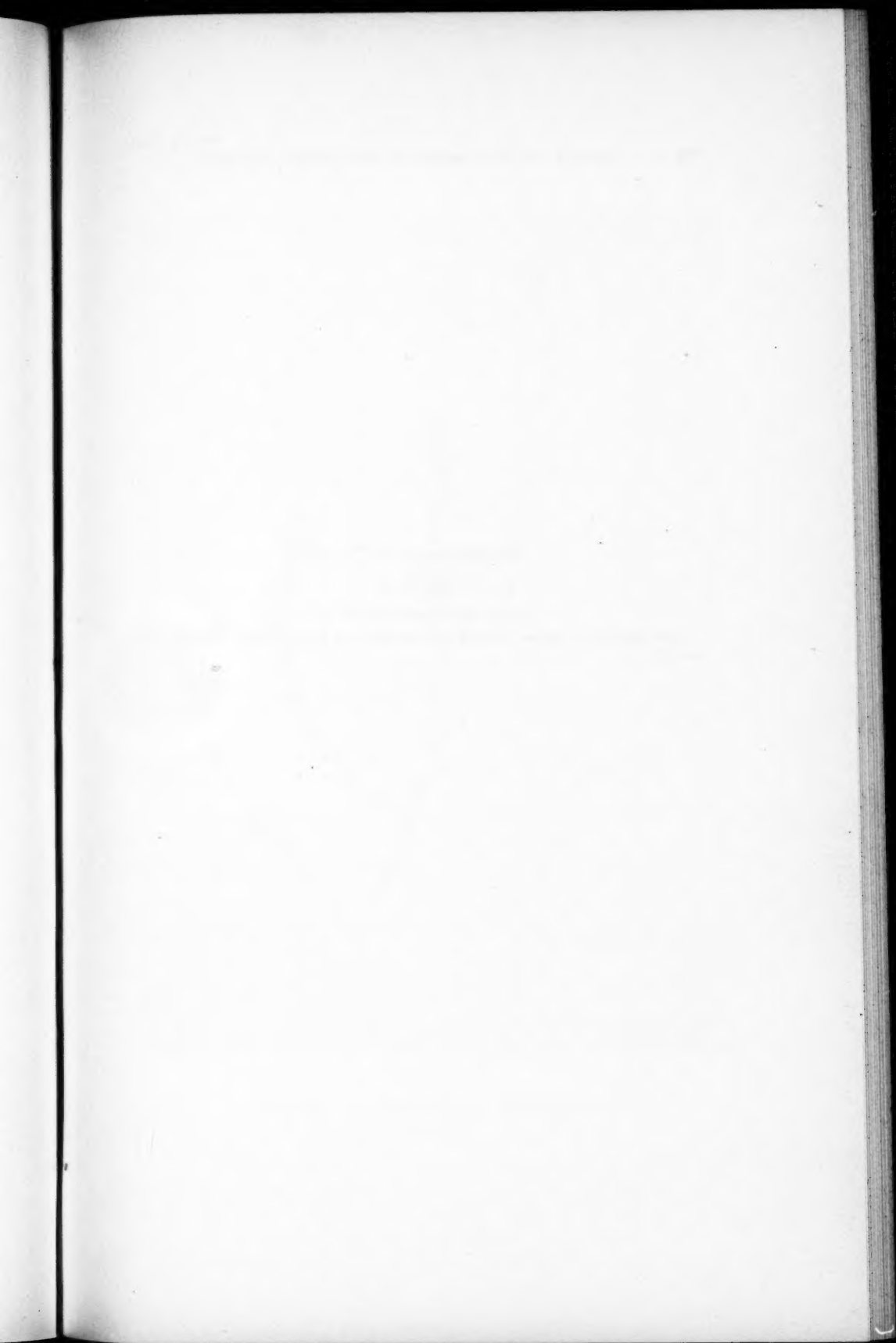
Dyschoriste xylopoda Kobuski

From the type specimen, *Pringle 4442*, in the herbarium of the Missouri Botanical Garden.



KOBUSKI—MONOGRAPH OF DYSCHORISTE





EXPLANATION OF PLATE

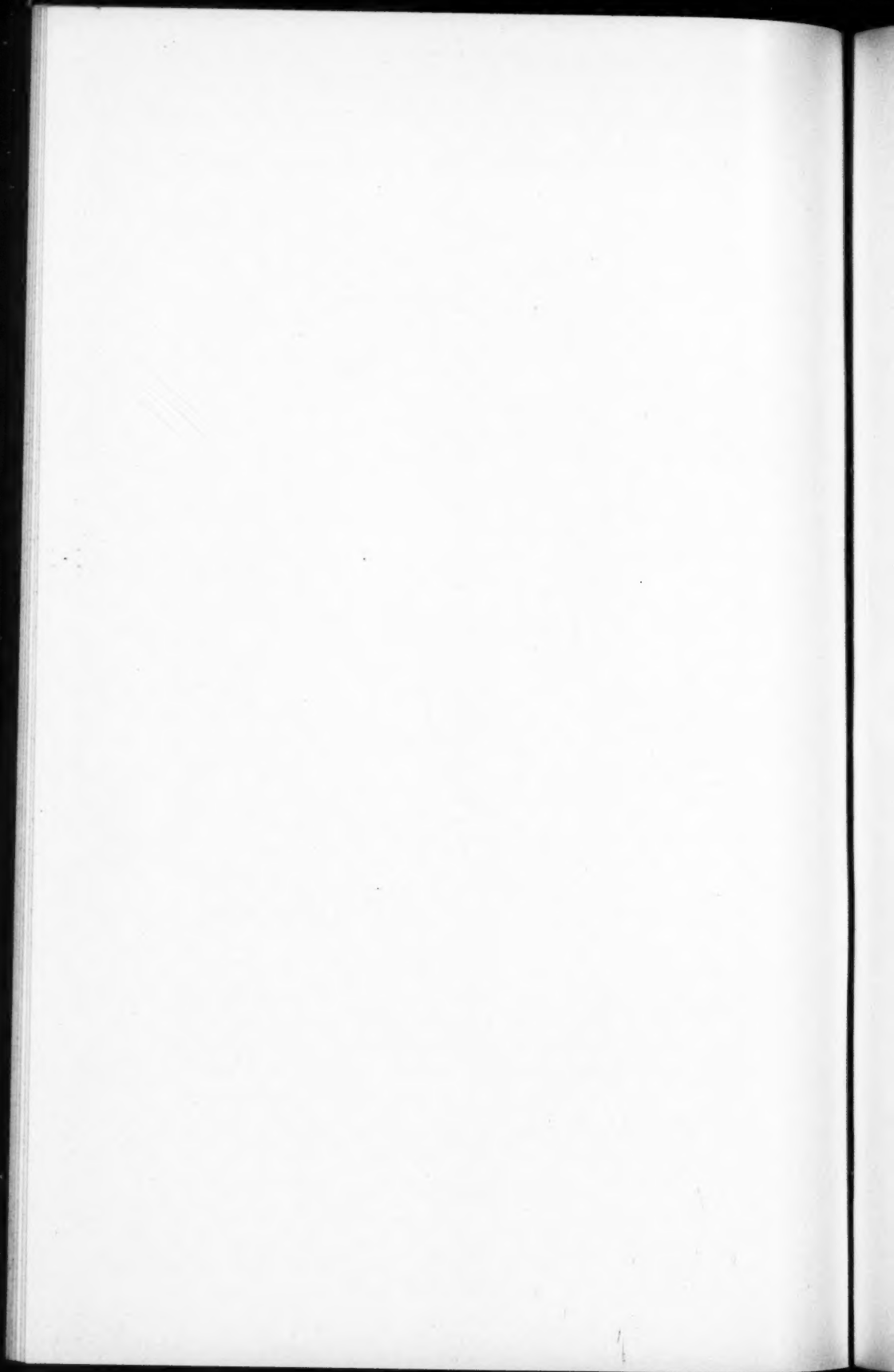
PLATE 16

Dyschoriste paraguariensis Kobuski

From the type specimen, Hassler 4355, in the Gray Herbarium of Harvard University.



KOBUSKI—MONOGRAPH OF DYSCHORISTE



STUDIES IN THE UMBELLIFERAE. I¹

MILDRED E. MATHIAS

Jessie R. Barr Research Fellow in the Henry Shaw School of Botany
of Washington University

A critical study of the genus *Cymopterus* has necessitated a detailed investigation of about twenty allied genera including *Glehnia* Schmidt.

Asa Gray in 1859 doubtfully referred a plant from the Cooper collection in the region of Puget Sound, Washington, to the genus *Cymopterus*, and in 1860 published the species as *Cymopterus* ? *littoralis*. Bentham ('67) in Bentham and Hooker's 'Genera Plantarum,' published in September, 1867, transferred this species to his new genus *Phellopterus*. F. Schmidt ('67), some time between January and July inclusive of the year 1867, in his 'Prolusio Florae Japonicae' published the new genus *Glehnia*² with one species, *G. littoralis*, basing it on a Maximowicz plant from Hakodate. In this work Schmidt also included *Cymopterus* ? *littoralis* Gray as a distinct species. In 1868 in his 'Flora Sachalinensis' he recognized that his *Glehnia littoralis* was conspecific with *Cymopterus* ? *littoralis* Gray and adopted the generic name *Phellopterus* of Bentham. Upon critical examination of the Cooper and Maximowicz types Schmidt's view as to the congeneric nature of the two is confirmed. As the generic name *Glehnia* of Schmidt was published at least two months prior to the *Phellopterus* of Bentham, on the basis of priority, it must be retained as the correct name for the genus and the Maximowicz plant must be taken as the generic type. The historical type of the genus is then the plant collected by Maximowicz in Hakodate, Japan, in 1861 and must bear the specific name *Glehnia littoralis* Schmidt.

Bentham, G. ('67). In Bentham, G., and J. D. Hooker, *Genera Plantarum* 1: 905. September, 1867.

Gray, Asa ('59). "Botany of Japan." *Mem. Am. Acad. N.S.* 6²: 391, 428. 1859.

—, ('60). Stevens' Report of U.S. Explorations & Surveys from the Mississippi River to the Pacific Ocean 12²: 62. 1860.

¹ Issued April 30, 1928.

² The genus *Glehnia* was so named in honor of Peter von Glehn who collected with Schmidt on the Island of Sachalin.

- Schmidt, F. ('67). *Prolusio Florae Japonicae* in Miq. Ann. Mus. Bot. Lugd. Bat. 3: 61. January-July, 1867; *Prolusio Florae Japonicae*, 249. 1867.
 ———, ('68). *Flora Sachalinensis*. Mem. Acad. Imp. Sci. St. Petersburg, VII, 12^e: 138-140. 1868.

Glehnia Schmidt, Prol. Fl. Jap. in Miq. Ann. Mus. Bot. Lugd. Bat. 3: 61. Jan.-July, 1867; Prol. Fl. Jap. 249. 1867; Baillon, Hist. Plant. 7: 215. 1880; Coult. & Rose, Contr. U. S. Nat. Herb. 7: 165. 1900; Piper, Contr. U. S. Nat. Herb. 11: 429. 1906; Henry, Fl. S. Brit. Col. 223. 1915; Piper & Beattie, Fl. N. W. Coast, 267. 1915; Carter & Newcombe, Prel. Cat. Fl. Vanc. 61. 1921.

Phellopterus Benth. in Benth. & Hook. Gen. Pl. 1: 905. September, 1867, not *Phellopterus* Nutt. (section under *Cymopterus* in Torr. & Gray, Fl. N. Am. 1: 623. 1840) in Coult. & Rose, Contr. U. S. Nat. Herb. 7: 166. 1900; Schmidt, Mem. Acad. Imp. Sci. St. Petersburg, VII, 12^e: 138. 1868; Franchet & Savatier, Enum. Plant. Jap. 1: 185. 1875; Wats. Bibl. Ind. 1: 430. 1878; Franchet, Cat. Plantes, in Mem. Soc. Nat. Sci. Cherbourg 24: 221. 1884; Coult. & Rose, Rev. N. Am. Umbell. 21, 81. 1888; Macoun, Check List Can. Plants, 25. 1889; Cat. Can. Plants 5: 329. 1890; Howell, Fl. N. W. Am. 1: 259. 1898; Engl. & Prantl, Nat. Pflanzenfam. 3^e: 221. 1898; Ito & Matsumura, Tent. Fl. Lutch. in Jour. Coll. Sci. Imp. Univ. Tokyo 12: 529. 1899; Yabe, Rev. Umb. Jap. in *Ibid.* 16^e: 92. 1902; Boiss. Omb. Cor. in Bull. Herb. Boiss. II, 3: 955. 1903; Nakai, Fl. Kor. 1 in Jour. Coll. Sci. Imp. Univ. Tokyo 26^e: 272. 1909.

Herbaceous, subcaulescent, glabrous or pubescent perennials. Leaves coriaceous, petioled, bipinnatisect, broadly ovate in general outline. Inflorescence pedunculate, villous, peduncles shorter than or equalling the leaves; involucre usually absent, sometimes present in the form of a few linear bracts; involucler of conspicuous linear-lanceolate bracts. Calyx teeth inconspicuous. Stylopodium lacking. Fruit ovate-oblong to globose, glabrous or pubescent, flattened dorsally; lateral and dorsal wings present; wings broadened at the base; oil-tubes large, numerous, 2-6 on the commissural side; strengthening cells absent.

Type species: *Glehnia littoralis* Schmidt, Prol. Fl. Jap. in Miq. Ann. Mus. Bot. Lugd. Bat. 3: 61. 1867; Prol. Fl. Jap. 249. 1867.

ABBREVIATIONS

The following abbreviations have been used in citations to indicate the different herbaria from which material has been obtained for study:

M = Missouri Botanical Garden Herbarium; G = Gray Herbarium of Harvard University; NY = New York Botanical Garden Herbarium; US = United States National Herbarium; W = Herbarium of the University of Washington deposited in the Washington State Museum; O = Herbarium of the University of Oregon; OAC = Herbarium of the Oregon Agricultural College; C = Herbarium of the University of California; P = Herbarium of Pomona College.

KEY TO THE SPECIES

Fruit pubescent, species of the eastern hemisphere.....1. *G. littoralis*
Fruit essentially glabrous, species of the western hemisphere.....2. *G. leiocarpa*

1. *Glehnia littoralis*¹ Schmidt, Prol. Fl. Jap. in Miq. Ann. Mus. Bot. Lugd. Bat. 3: 61. 1867; Prol. Fl. Jap. 249. 1867.

Pl. 17, fig. 2, 3, 5; Pl. 18; Pl. 19, fig. 1.

"*Archangelica officinalis*, Hoffm.?" in Gray, "Account of the Botanical Specimens" from Narrative of the Perry Expedition 2: 312. 1856.

"*Cymopterus* (?) *littoralis*, glaber" Gray, "Botany of Japan," in Mem. Am. Acad. N. S. 6²: 428. 1859, *nomen nudum*.

Cymopterus ? *littoralis* Gray, "Botany of Japan" in Mem. Am. Acad. N. S. 6²: 391, 428. 1859, as to specimens from eastern hemisphere, *nomen nudum*.

"*Cymopteris glaber* (A. Gray)" Black, "Catalogue of Japan

¹ *Glehnia littoralis* Schmidt, em.—Planta humila, subacaula; foliis, petiolis excludentis, 5-13 cm. longis latisque, supra hirtellis in rachides nervosque, subtus glabris vel crebre tomentosis, ultimis segmentis foliorum oblongo-obovatis vel segmentis terminalibus cuneatis, 0.5-5 cm. longis, 0.4-4 cm. latis, apice rotundatis vel acutis, plus minusve cartilagineo-dentatis; petiolis 3-12 cm. longis, subdilatis, hirtellis vel glabris; inflorescentiis umbellatis pedunculatis, crebre villosis; pedunculis subcrassis, subinde ramosis, foliis brevioribus vel aequantibus; umbellis patulis, 6-30-radiatis, radiis 1-3.5 cm. longis; involuero 1-3-bracteato; umbellulis capitatis, bracteis involucellorum pluribus, lanceolato-attenuatis; fructibus ovato-oblongis vel subglobosis, 0.4-1.5 cm. longis, villosopubescentibus, pilis multicellulatis; alis lateralibus saepe dorsalibus latioribus; vittis multis, 2-6 in commissuram.—Collected in Hakodate, Japan, 1861, *Mazimowicz* (Gray Herb.), CO-TYPE.

Plants" in Hodgson, "A residence at Nagasaki and Hakodate in 1859-1860," 335. 1861, *nomen nudum*; Bonplandia 10: 92. 1862, *nomen nudum*.

Phellopterus littoralis (Gray) Benth. in Benth. & Hook. Gen. Pl. 1: 905. 1867, as to plants of eastern hemisphere; Hance, Spic. Fl. Sin. in Jour. Bot. 16: 12. 1878; Forbes & Hemsley, Jour. Linn. Soc. Bot. 23: 331. 1888; Engl. & Prantl, Nat. Pflanzenfam. 3: 221. 1898, as to plants of eastern hemisphere; Ito & Matsumura, Tent. Fl. Lutch. in Jour. Coll. Sci. Imp. Univ. Tokyo 12: 529. 1899; Yabe, Rev. Umb. Jap. in *Ibid.* 16: 93. 1902; Boiss. Omb. Cor. in Bull. Herb. Boiss. II, 3: 955. 1903; Nakai, Fl. Kor. 1. in Jour. Coll. Sci. Imp. Univ. Tokyo 26: 272. 1909; Fl. Kor. 2. in *Ibid.* 31: 492. 1911.

"*C. glaber*" Gray acc. to Schmidt, Mem. Acad. Imp. Sci. St. Petersburg, VII, 12: 139. 1868, *nomen nudum*.

"*Phellopterus littoralis*" acc. to Schmidt, Mem. Acad. Imp. Sci. St. Petersburg, VII, 12: 138. 1868.

"*Phellopterus littoralis* Schmidt" acc. to Franchet & Savatier, Enum. Plant. Jap. 1: 185. 1875; Franchet, Cat. Plantes, in Mem. Soc. Nat. Sci. Cherbourg 24: 221. 1884.

"*Glehnia littoralis* (Gray) Schmidt" acc. to Coult. & Rose, Contr. U. S. Nat. Herb. 7: 165. 1900, as to plants of eastern hemisphere.

Low subcaulescent plants; leaves, excluding petiole, 5-13 cm. long, about as broad, hirtellous on the rachises and nerves of the upper surface, glabrous to densely tomentose beneath, the ultimate leaf-segments oblong-obovate or the terminal segments cuneate, 0.5-5 cm. long, 0.4-4 cm. broad, rounded to acute at the apex, somewhat unequally cartilaginously dentate; petioles 3-12 cm. long, somewhat inflated, hirtellous to glabrous; inflorescence pedunculate, densely villous; peduncles stoutish, sometimes branched, shorter than or equalling the leaves; umbels spreading, 6-30-rayed, rays 1-3.5 cm. long; involucre 1-3-bracted; umbellets capitate, bracts of the involucre several, lance-attenuate; fruit ovate-oblong to subglobose, 0.4-1.5 cm. long, villous-pubescent with multicellular hairs, lateral wings usually broader than the dorsal wings; oil-tubes numerous, 2-6 on the commissural surface.

Type specimen: *Maximowicz*, "Glehnia littoralis F. Schmidt. Fl. Sachalin ined." Iter secundum. Japonia. Hakodate. 1861. (TYPE probably in Herb. Leningrad; CO-TYPE in the Gray Herbarium of Harvard University).

Distribution: eastern hemisphere, along sandy sea-shores, from southern China northward, and in Japan.

This plant is commonly known in Japan as "*Hama-bofu*" in relation to its maritime habitat, *hama* meaning sea-coast, and *bofu*, a medicinal plant.

Specimens examined:

JAPAN: Insula Sachalin, 1860, *Schmidt* (G); Kamiiso, Prov. Oshima, Hokkaido, 12 July, 1890, *Miyabe & Tokubuchi* (G); Hakodate, Iter secundum, 1861, *Maximowicz* (G CO-TYPE); Insula Jesso, circa Hakodate, 1861, *Albrecht* (G); Yezo, Ishikari, 10 Sept. 1903, *Arimoto* (G, M); Nambu, Nippon, 1865, *Maximowicz*, coll. Tschonoski (NY); Isoya, Shiribeshi, July, 1883, *Take-nobu* (G); seashore, Prov. Rikuzen, 9 July, 1913, *Yasuda* (W); Isl. Futami, 24 June, 1910, Flora Japonica, collector unknown (US 1155343); Loo-Choo Islands, 1853-56, *Wright 98* (G, US); Corea, 1859, *Wilford* (NY).

SIBERIA: Vladivostok and vicinity, May-Oct. 1919, *Topping 2236* (G).

CHINA: Tsingtao, 1911, *Zimmermann* (G, US 795348); "Putoo Island—Clekiang," *Henry* (M); "Pootoo Isle, Chekiang," *Faber M^e* (US); Delatache and Amoy, *Henry* (NY).

2. *Glehnia leiocarpa*¹ Mathias, nom. nov.

Pl. 17, fig. 1, 4; Pl. 19, fig. 2.

Cymopterus ? *littoralis* Gray, Mem. Am. Acad. N. S. 6²: 391, 428. 1859, as to American specimens, *nomen nudum*; Stevens' Rept. U. S. Expl. & Surv. from Miss. to Pacific Ocean 12²: 62. 1860; Jeps. Man. Fl. Plants Calif. 731. 1925.

Phellopterus littoralis (Gray) Benth. in Benth. & Hook. Gen.

¹ *Glehnia leiocarpa* Mathias, nom. nov.—Planta humilis, subacaula; foliis, petiolis excludentis, 2.5-15 cm. longis latisque, supra hirtellis in rachides nervosque, subtus crebre tomentosis, ultimis segmentis foliorum oblongo-obovatis vel segmentis terminalibus cuneatis, 0.5-5 cm. longis, 0.4-3 cm. latis, apice rotundatis vel acutis, plus minusve dentatis, marginibus subinde cartilaginibus; petiolis 2.5-14 cm. longis, subdilatatis, hirtellis; inflorescentiis umbellatis pedunculatis, crebre villosis; pedunculis subcrassis, subinde ramosis, saepe foliis brevioribus, rare aequantibus; umbellis

Pl. 1: 905. 1867, as to American plants; Engl. & Prantl, Nat. Pflanzenfam. 3^a: 221. 1898, as to American plants.

"*Glehnia littoralis* (Gray) Schmidt" acc. to Coult. & Rose, Contr. U. S. Nat. Herb. 7: 165. 1900; Piper, Contr. U. S. Nat. Herb. 11: 429. 1906; Piper & Beattie, Fl. N. W. Coast, 267. 1915; Carter & Newcombe, Prel. Cat. Fl. Vanc. 61. 1921.

"*Phellopterus littoralis* Schmidt" acc. to Wats. Bibl. Ind. 1: 430. 1878; Coult. & Rose, Rev. N. Am. Umbell. 81. 1888; Macoun, Check List Can. Plants, 25. 1889; Cat. Can. Plants 5: 329. 1890; Howell, Fl. N. W. Am. 1: 259. 1898.

Glehnia littoralis Schmidt acc. to Henry, Fl. S. Brit. Col. 223. 1915.

Low subacaulescent plants; leaves, excluding petiole, 2.5–15 cm. long, about as broad, hirtellous on the rachises and nerves of the upper surface, mostly densely tomentose beneath, the ultimate leaf-segments oblong-obovate or the terminal segments cuneate, 0.5–5 cm. long, 0.4–3 cm. broad, rounded to acute at the apex, unequally dentate, margins sometimes cartilaginous; petioles 2.5–14 cm. long, somewhat inflated, hirtellous; inflorescence pedunculate, densely villous; peduncles stoutish, sometimes branched, usually shorter than the leaves, rarely equalling them; umbel globose to spreading, 5–13-rayed, rays 0.5–4.5 cm. long; involucre 1–3-bracted; umbellets capitate, bracts of the involucre several, lance-attenuate; fruit ovate-oblong to subglobose, 0.4–1.2 cm. long, essentially glabrous (sometimes with a few scattered multicellular hairs), lateral wings sometimes broader than dorsal wings; oil-tubes numerous, 2–6 on commissural surface.

Type specimen: *J. G. Cooper*, "sandy shores, Washington Terr. (Shoal Water Bay)." 1854. (The type is in the Gray Herbarium of Harvard University and is labeled "*Cymopterus ? littoralis*, n. sp." in Gray's handwriting; co-types are in the Herbarium of the New York Botanical Garden and in the United States National Herbarium.)

globosis vel patulis, 5–13-radiatis, radiis 0.5–4.5 cm. longis; involucre 1–3-bracteato; umbellulis capitatis, bracteis involucellorum pluribus, lanceolato-attenuatis; fructibus ovato-oblongis vel subglobosis, 0.4–1.2 cm. longis, fere glabris vel subinde sparse pubescentibus, pilis multicellulatis; alis lateralibus dorsalibus latioribus; vittis multis, 2–6 in commissuram.—Collected on sandy shores, Shoal Water Bay, Washington Territory (State of Washington), 1854, *J. G. Cooper* (Gray Herb.), TYPE.

Distribution: North America along sandy sea-coasts from San Francisco, California northward.

Specimens examined:

ALASKA: along the Ankow River, near Ocean Cape, vicinity of Yakutat Bay, 1 July, 1892, *Funston 51* (NY, M, C).

BRITISH COLUMBIA: vicinity of Ucleulet, Long Beach, Vancouver Island, 25 June, 1909, *Macoun 78600* (US); sand, Oak Bay, Vancouver Island, 31 May, 1887, *Macoun* (G).

WASHINGTON: Lopez, San Juan Islands, 25 June–1 Aug. 1917, *S. M. & E. B. Zeller 963* (NY, M, G, US); Puget Sound, *Wilkes Expedition* (NY, US 44092); Port Angeles, 26 June, 1908, *Webster* (W); sand dunes, Ocean Park, April, 1908, *Rigg* (W); Ilwaco, 21 June, 1904, *Piper 5002* (US); Oyhut, Chehalis County, 7 June, 1897, *Lamb 1249* (NY, M); drifting sand, common along the ocean beach, Westport, Chehalis Co., 26 June, 1892, *Henderson 385* (US); ocean beach, Westport, Chehalis County, 26 June, 1892, *Henderson* (W); sand dunes, Westport, June, 1917, *Grant* (NY); sand spit, Sequim, June, 1915, *Grant* (NY, M 788926); Seattle, July, 1915, *Freiberg* (M 813695); sandy dunes, mouth of "Joe Creek," near Moclips, 28 June, 1908, *Foster 824* (US); sandy sea-shores, Port Angeles, 26 June, 1908, *Flett 3375* (US); Olympic Mts., Clallam Co., July, 1900, *Elmer 2768* (NY, M, US); M. Beach, Westport, 10 July, 1907, *Cowles 512* (M); "sandy shores, Washington Terr. (Shoal Water Bay)." 1854, *Cooper* (G TYPE, NY, US); beach sand, Copalis, June–July, 1902, *Conrad 392* (US); Copalis, 30 May, 1912, *Bardell* (M 813656).

OREGON: Clatsop Beach, Clatsop Co., 21 Aug. 1902, *Sheldon 11252* (NY, M, G, P, US); Gearhart, 19 June, 1904, *Piper 6241* (US); Gearhart, 19 June, 1904, *Piper 6131* (US); sandy sea-beach, Newport, 3 July, 1918, *J. Nelson 2292* (G); Nestart's Bay, Tillamook Co., 29 June, 1894, *Lloyd* (NY); on strand, Nestucca, July–Aug. 1901, *Kirkwood 149* (NY); sandy sea-shore, mouth of the Umpqua River, 18 June, 1885, *Howell*¹ (OAC, US, M, 1151); on sand dunes, mouth of Tillamook Bay, 16 July, 1882, *T. Howell* (NY); on shifting sand, Tillamook Bay, 14 July,

¹ Thomas Howell in the earlier period of his botanical career used the signature Thomas J. Howell which accounts for the discrepancy in names appearing on herbarium labels.

1882, *T. J. Howell* (M, US 33339); Clatsop Beach, 26 July, 1891, *J. Howell* (M 863104); on shifting sands of sea-shore, Coos Bay, 19 Aug. 1911, *House 4705* (US, NY); drifting sand, ocean beach, Tillamook Bay, 14 July, 1882, *Howell & Henderson* (O); drifting sand, ocean beach, Clatsop, 30 July, 1887, *Henderson 385* (M, OAC); beach, below Florence, 20 May, 1925, *Henderson* (O); sand of the ocean above high tide, Rockaway, 16 Sept. 1925, *Henderson* (O); sea-shore, Fort Stevens, 7 July, 1886, *Henderson* (O); Bayocean, Garibaldi, 28 Aug. 1914, *Hitchcock 12370* (US); sands of the Oregon coast between Umpqua and Coos Bay, 12 Aug. 1880, *G. Engelmann* (M); on sand dunes of the ocean, Gearhart, Clatsop County, 1 Sept. 1898, *Coville 861* (US).

CALIFORNIA: in drifting sand, Humboldt County, sand hills of ocean beach at Samoa, opp. Eureka, 7 Aug. 1901, *Tracy 1261* (C); Samoa Beach, Humboldt Co., 17 June, 1911, *Smith 3854* (NY); Trinidad, Humboldt Co., 7 June, 1911, *Smith 3806* (NY); Trinidad, Humboldt County, 6 July, 1911, *Smith 3806* (US); Pebble Beach, Crescent, Del Norte Co., 17-20 June, 1925, *Parks 8257* (C 279023); sandy dunes at Humboldt County camp, 7 miles south of Trinidad, 24 July, 1924, *A. A. Heller 13382* (NY); Crescent City, Del Norte Co., 30 June, 1899, *Davy & Blasdale 5960* (C); Point Arena, Mendocino Co., 24 July, 1900, *Davy 6050* (C); peninsula, Eureka, 23 Aug. 1904, *Congdon* (C 140694); sea-shore peninsula, Eureka, Humboldt Co., 23 July, 1904, *Congdon* (M); Humboldt Bay, May, 1901, *Chandler 1145* (C); Trinidad, Humboldt Co., 18 July, 1916, *Abrams 6140* (NY, O).

The genus *Glehnia* is characterized by its maritime habitat, broad leaf divisions, thick coriaceous texture of the leaves, and prominent wing development of the fruit. The two species are separated largely on fruit characters. *Glehnia littoralis* Schmidt, the species of the eastern hemisphere, always has a pubescent fruit. The pubescence is villous with multicellular hairs. The mature fruit may be only slightly pubescent due to the falling off of the hairs but in such cases it has a tuberculate appearance showing the previous attachment of these hairs. In the young fruit the pubescence is densely villous. As a rule the oil-tubes of the fruit are smaller and more numerous than in the other

species. The characters of inflorescence and foliage are similar in both species. There is quite a range of variation in foliage pubescence of *Glehnia littoralis*. The type of the species, the Maximowicz plant from Hakodate, represents an intermediate condition, and upon an examination of additional material may prove to be a hybrid between the glabrous and pubescent forms (pl. 18, fig. 2). The leaves are hirtellous on the lower surface and on the veins and rachises of the upper surface. The one extreme of variation in pubescence is typified by the plant collected by Wright in the Loo Choo Islands and labeled by Gray "Cymopterus littoralis ?? Gray, var. glabra, vel sp. aff." (pl. 18, fig. 1). The leaf is essentially glabrous, the hirtellous condition being limited entirely to the veins and rachises. The margins of the leaves are more frequently cartilaginous than in other forms. The other extreme of variation is typified by the plant collected by Schmidt in Sachalin in 1860 (pl. 19, fig. 1). This plant superficially more closely approaches the species of North America. The lower surface of the leaf has the same dense tomentose pubescence that occurs in *Glehnia leiocarpa*. However, an examination of a large amount of material from the eastern hemisphere shows a great number of intergrading forms; a gradual variation exists from the extreme glabrous form to the densely tomentose one. The fruit in all forms is similar, and the pubescence characters of the foliage are of no value in separating *Glehnia littoralis* into varieties or forms.

Glehnia leiocarpa, on the other hand, shows a very constant pubescence character. The leaves in every case are densely tomentose beneath. The fruit is glabrous with the exception of occasional multicellular hairs on the margins of the wings. In no case was a tuberculate appearance observed which would point to the previous attachment of hairs in younger conditions. The young fruit in most cases is essentially glabrous. Moreover, a cross-section of the fruit shows the oil-tubes to be larger and generally fewer in number than in *G. littoralis*.

An interesting geographical distribution is shown in connection with this genus. The two closely related species occur along the coast on both sides of the Pacific Ocean (fig. 1). *Glehnia leiocarpa* extends from Alaska to northern California and *G. littoralis* from

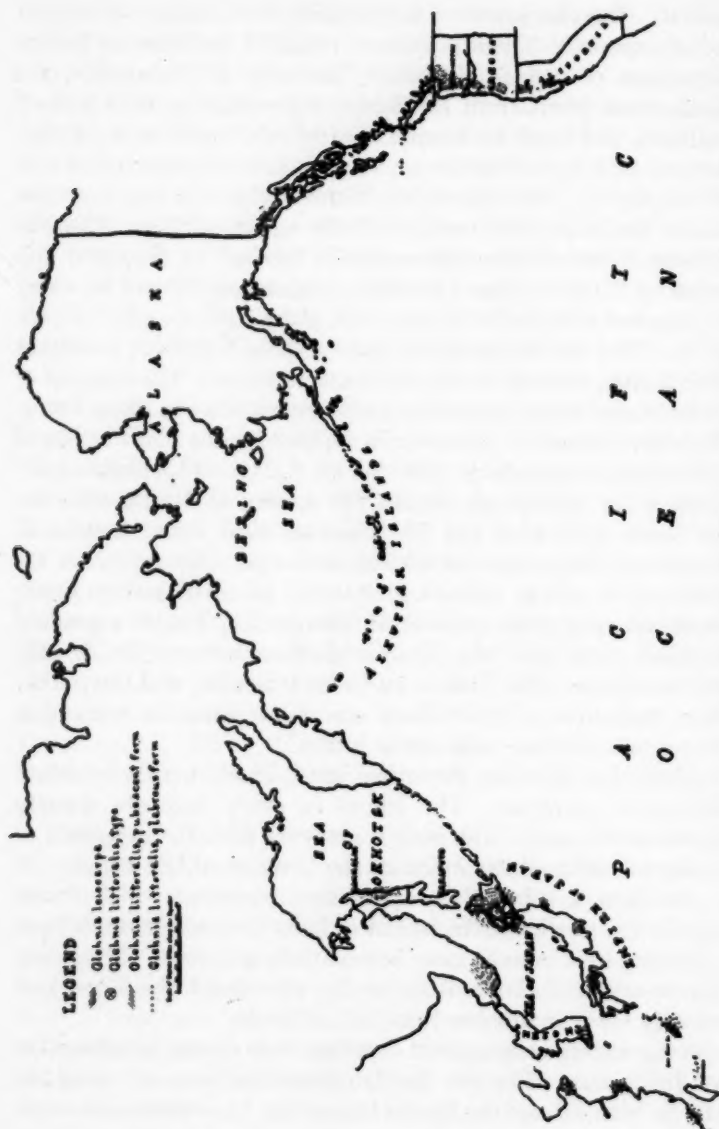


Fig. 1. Showing distribution of species of Glehnia.

Siberia to southern China and through Japan. It is also interesting to note the distribution of the different pubescence types of *G. littoralis*. The more pubescent plants and those most nearly approaching *G. leiocarpa* occur in the northern region of the distribution area of the species, while the more glabrous plants are found in the southern range of distribution. Such a distribution seems to indicate that the ancestors of this species occurred in the intermediate area and in the land bridge connecting North America and Asia somewhere in the Bering Sea region.

A similar distribution for other genera has been pointed out by various workers in this field. One of the earliest important works was Dr. Gray's¹ article on the "Botany of Japan" in which he showed the similarity of the flora of northwest as well as eastern America to that of Japan. In this work he also mentions the distribution of the genus *Glehnia*. Butters,² more recently, has pointed out a similar distribution for the genus *Athyrium*. Berry³ has shown this distribution for *Castanopsis*, *Pasania*, *Corylus*, *Juglans*, and other genera.

The writer is indebted to Dr. George T. Moore, Director of the Missouri Botanical Garden, for the use of the library and herbarium of that institution. Sincere appreciation is due Dr. N. L. Britton and Dr. J. K. Small of the New York Botanical Garden, Dr. B. L. Robinson and Dr. Ivan M. Johnston of the Gray Herbarium, Dr. Wm. R. Maxon of the United States National Herbarium, Prof. L. F. Henderson of the University of Oregon, Dr. Helen M. Gilkey of Oregon Agricultural College, Prof. T. C. Frye and Miss Martha R. Flahaut of the University of Washington, Dr. N. L. Gardner of the University of California, and Dr. Philip A. Munz of Pomona College for the privilege of examining material in the herbaria of the above-mentioned institutions or for the loan of material necessary for this study. Thanks are also due Dr. John H. Barnhart of the New York Botanical

¹ Gray, A. "Botany of Japan." Mem. Am. Acad. N.S. 6²: 376-449. 1859.

² Butters, F. K. Taxonomic and geographic studies in North American ferns. I. The genus *Athyrium* and the North American ferns allied to *Athyrium Filix-femina*. Rhodora 19: 169-207. 1917.

³ Berry, E. W. Tree ancestors. 270 pp. 1923.

Garden, Dr. J. N. Rose of the United States National Herbarium, and Dr. F. A. F. C. Went of the Botanical Laboratory of Utrecht for their assistance in bibliographical details. Especial thanks are due Dr. J. M. Greenman, Curator of the Herbarium of the Missouri Botanical Garden, for his advice and assistance.

LIST OF EXSICCATAE

The distribution numbers are printed in *italics*. The number in parenthesis is the species number used in this revision.

- | | |
|--|---|
| Abrams, L. <i>6140</i> (2). | Howell, J. — (2). |
| Albrecht, N. — (1). | Howell, T. — (2). |
| Arimoto, S. — (1). | Howell, T. J. — (2). |
| Bardell, E. M. — (2). | Kirkwood, J. E. <i>149</i> (2). |
| Chandler, H. P. <i>1145</i> (2). | Lamb, F. H. <i>1248</i> (2). |
| Congdon, J. W. — (2). | Lloyd, F. E. — (2). |
| Conrad, H. S. <i>392</i> (2). | Macoun, J. —, 78600 (2). |
| Cooper, J. G. — (2). | Maximowicz, C. J. — (1). |
| Coville, F. V. <i>861</i> (2). | Maximowicz, C. J. (coll. Tschonoski) — (1). |
| Cowles, H. C. <i>512</i> (2). | Miyabe, K. and Tokubuchi, E. — (1). |
| Davy, J. B. <i>6050</i> (2). | Nelson, J. C. <i>2292</i> (2). |
| Davy, J. B. and Blasdale, W. C. <i>5960</i> (2). | Parks, H. E. <i>8257</i> (2). |
| Elmer, A. D. E. <i>2768</i> (2). | Piper, C. V. <i>5002</i> , <i>6131</i> , <i>6241</i> (2). |
| Engelmann, G. — (2). | Rigg, G. B. — (2). |
| Faber, E. <i>M^a</i> (1). | Schmidt, F. — (1). |
| Flett, J. B. <i>3375</i> (2). | Sheldon, E. P. <i>11252</i> (2). |
| Fl. Japonica (collector unknown), — (1). | Smith, H. H. <i>3806</i> , <i>3854</i> (2). |
| Foster, A. S. <i>824</i> (2). | Takenobu, S. — (1). |
| Freiberg, G. W. — (2). | Topping, L. <i>2236</i> (1). |
| Funston, F. <i>51</i> (2). | Tracy, J. P. <i>1261</i> (2). |
| Grant, J. M. — (2). | Webster, E. B. — (2). |
| Heller, A. A. <i>13882</i> (2). | Wilford, C. — (1). |
| Henderson, L. F. — <i>385</i> (2). | Wilkes Expedition, — (2). |
| Henry, A. — (1). | Wright, C. <i>98</i> (1). |
| Hitchcock, A. S. <i>12370</i> (2). | Yasuda, A. — (1). |
| House, H. D. <i>4705</i> (2). | Zeller, S. M. and E. B. <i>963</i> (2). |
| Howell T. and Henderson, L. F. — (2). | Zimmermann, R. — (1). |

INDEX OF SPECIES

New species and combinations are printed in **bold face** type; synonyms in *italics*; and previously published names in ordinary type.

" <i>Archangelica officinalis</i> Hoffm.?"	93	Glehnia leiocarpa Mathias	
Athyrium	101	93, 95, 99, 101
" <i>C. glaber</i> " Gray	94	Glehnia littoralis Schmidt	
Castanopsis	101	91, 92, 93, 95, 96, 98, 99, 101
Corylus	101	" <i>Glehnialittoralis</i> (Gray) Schmidt"	
" <i>Cymopteris glaber</i> (A. Gray)"		94, 96
Black	93	Juglans	101
<i>Cymopterus</i> Raf.	91, 92	Pasania	101
<i>Cymopterus</i> sect. <i>Phellopterus</i> Nutt.	92	<i>Phellopterus</i> Benth.	91, 92
<i>Cymopterus?</i> <i>littoralis</i> Gray	91, 93, 95	<i>Phellopterus</i> Nutt.	92
" <i>Cymopterus littoralis?</i> " Gray var.		<i>Phellopterus littoralis</i> (Gray) Benth.	
<i>glabra</i>	99	94, 95
" <i>Cymopterus</i> (?) <i>littoralis glaber</i> "		" <i>Phellopterus littoralis</i> Schmidt"	94, 96
Gray	93	" <i>Phellopterus littoralis</i> " acc. to	
Glehnia Schmidt	91, 98, 101	Schmidt	94

EXPLANATION OF PLATE

PLATE 17

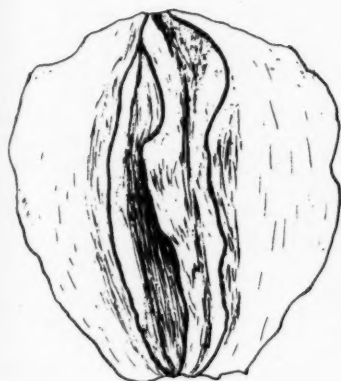
Fig. 1. Mature fruit of *Glehnia leiocarpa* Mathias, collected on "sandy shores, Washington Terr. (Shoal Water Bay)," Cooper, 1854 (Gray Herb.), TYPE. $\times 6$.

Fig. 2. Mature fruit of *Glehnia littoralis* Schmidt, collected on the Island of Sachalin, Schmidt, 1860 (Gray Herb.). $\times 6$.

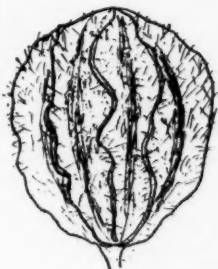
Fig. 3. Mature fruit of *Glehnia littoralis* Schmidt, collected in Yezo, Ishikari, Arimoto, 10 Sept. 1903 (Mo. Bot. Gard. Herb.). $\times 6$.

Fig. 4. Cross-section in median plane of immature fruit of *Glehnia leiocarpa* Mathias, collected on "sandy shores, Washington Terr. (Shoal Water Bay)," Cooper, 1854 (Gray Herb.), TYPE. $\times 10$.

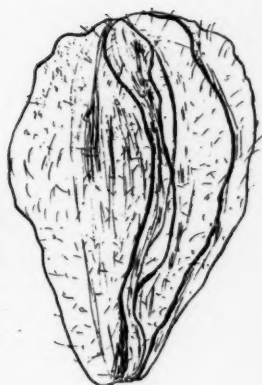
Fig. 5. Cross-section in median plane of mature fruit of *Glehnia littoralis* Schmidt, collected on the Island of Sachalin, Schmidt, 1860 (Gray Herb.). $\times 10$.



1



2



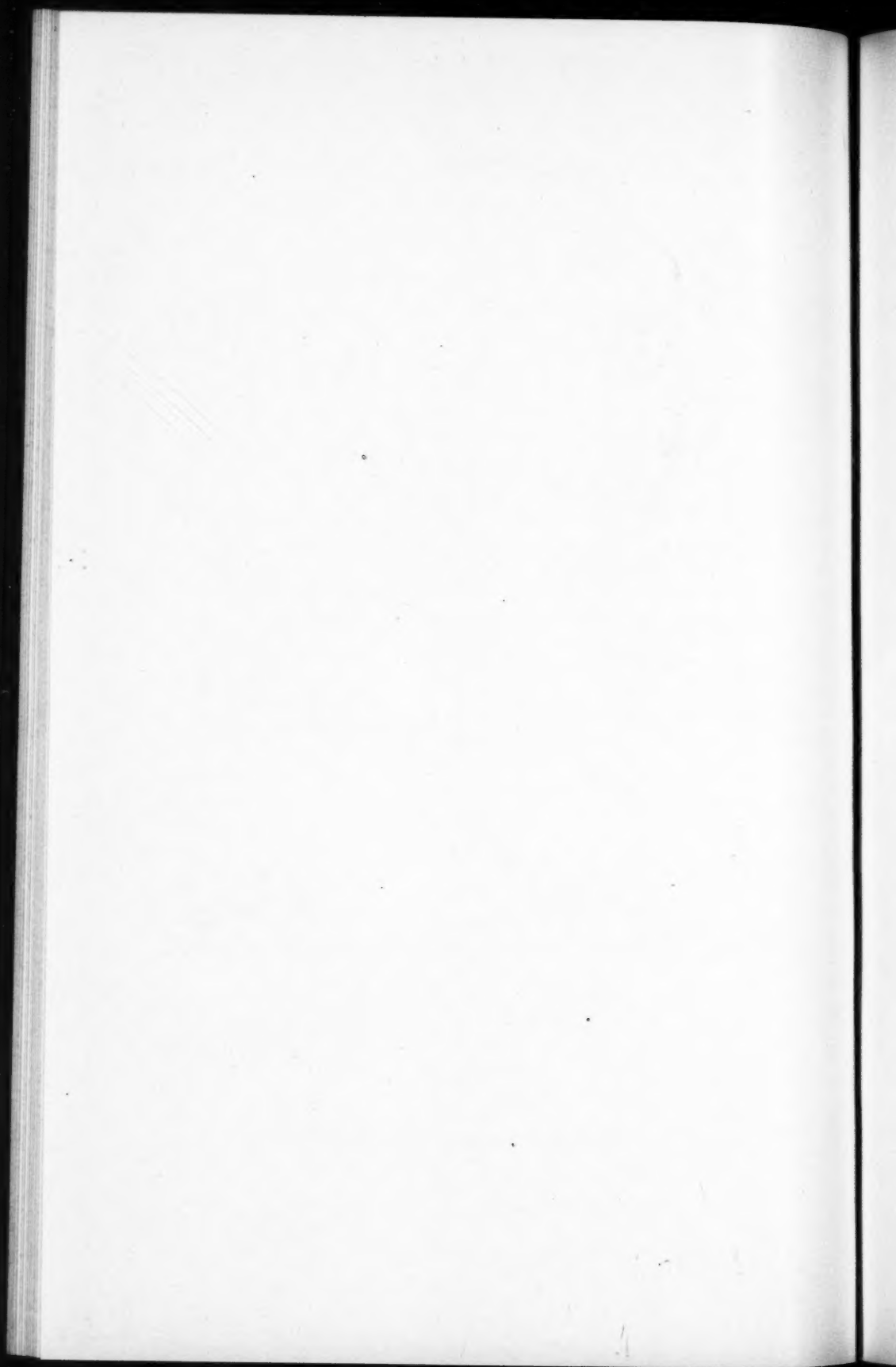
3

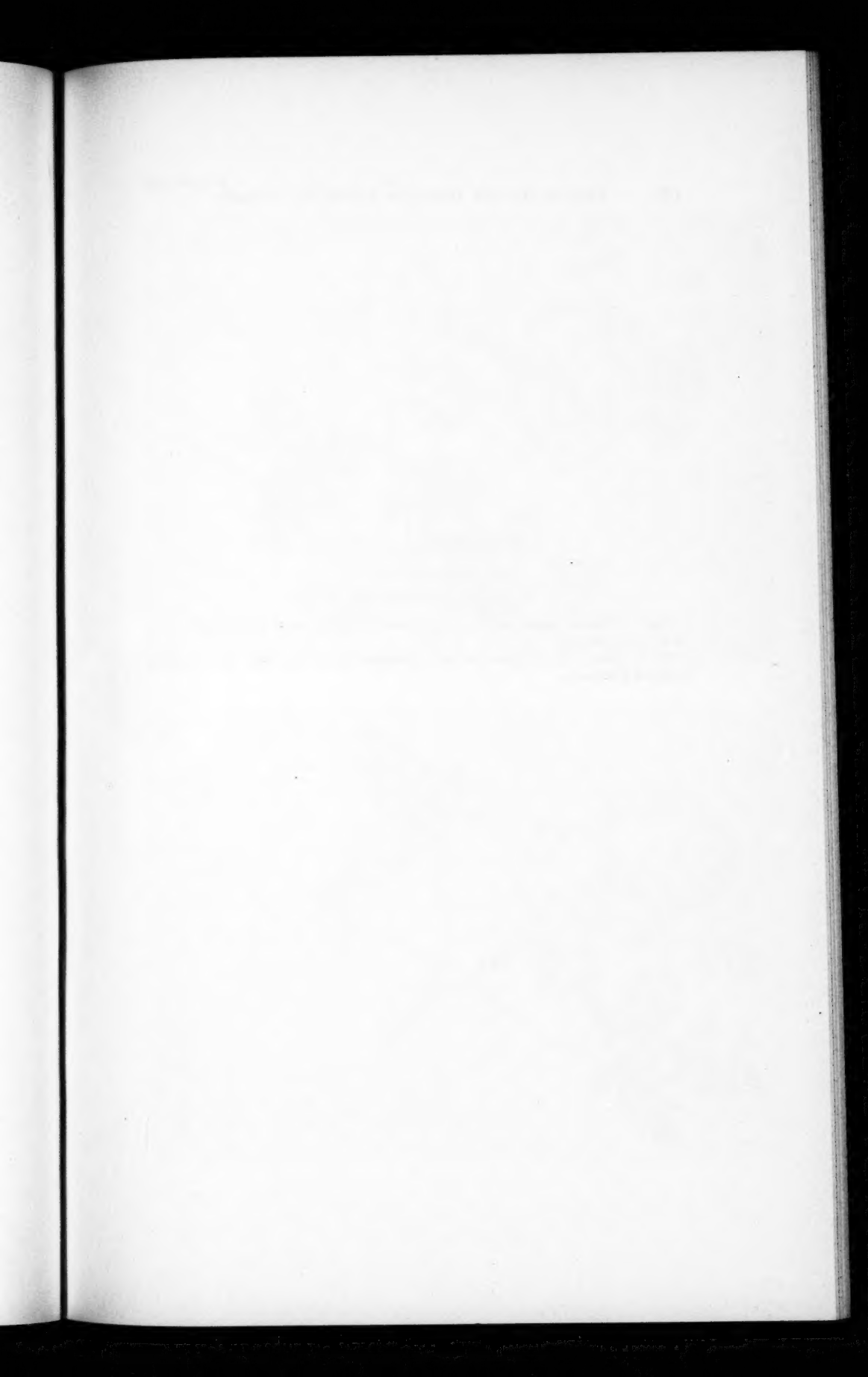


4



5





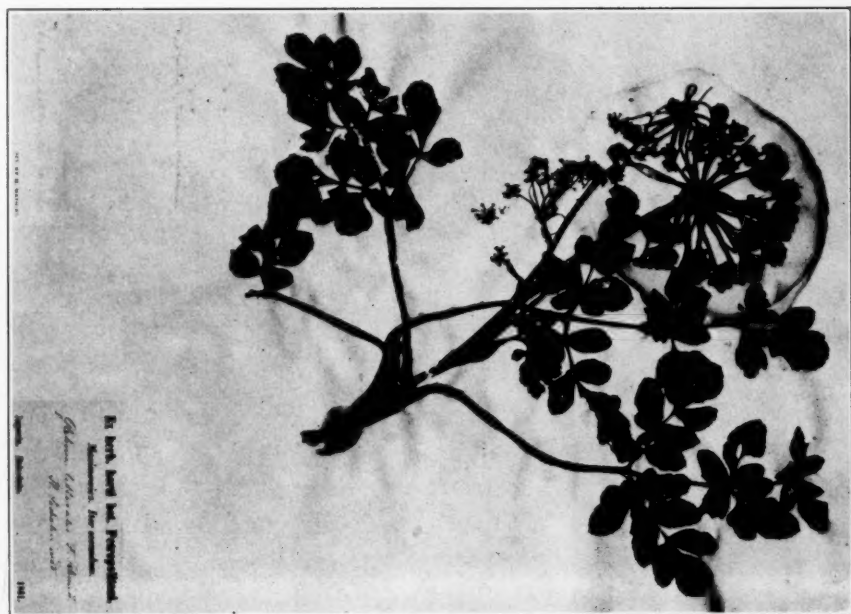
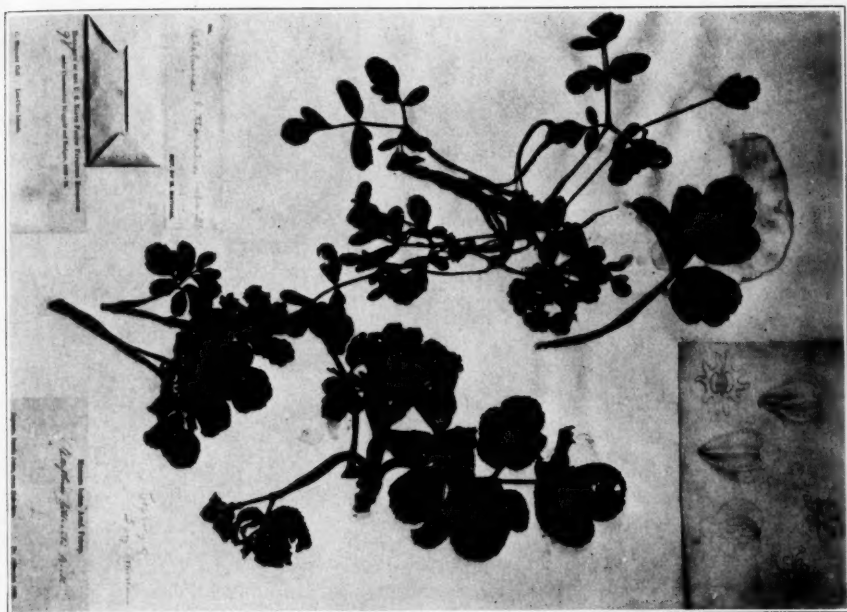
EXPLANATION OF PLATE

PLATE 18

Glehnia littoralis Schmidt

Fig. 1. From specimens in the Gray Herbarium of Harvard University, namely *Wright* and *Albrecht*.

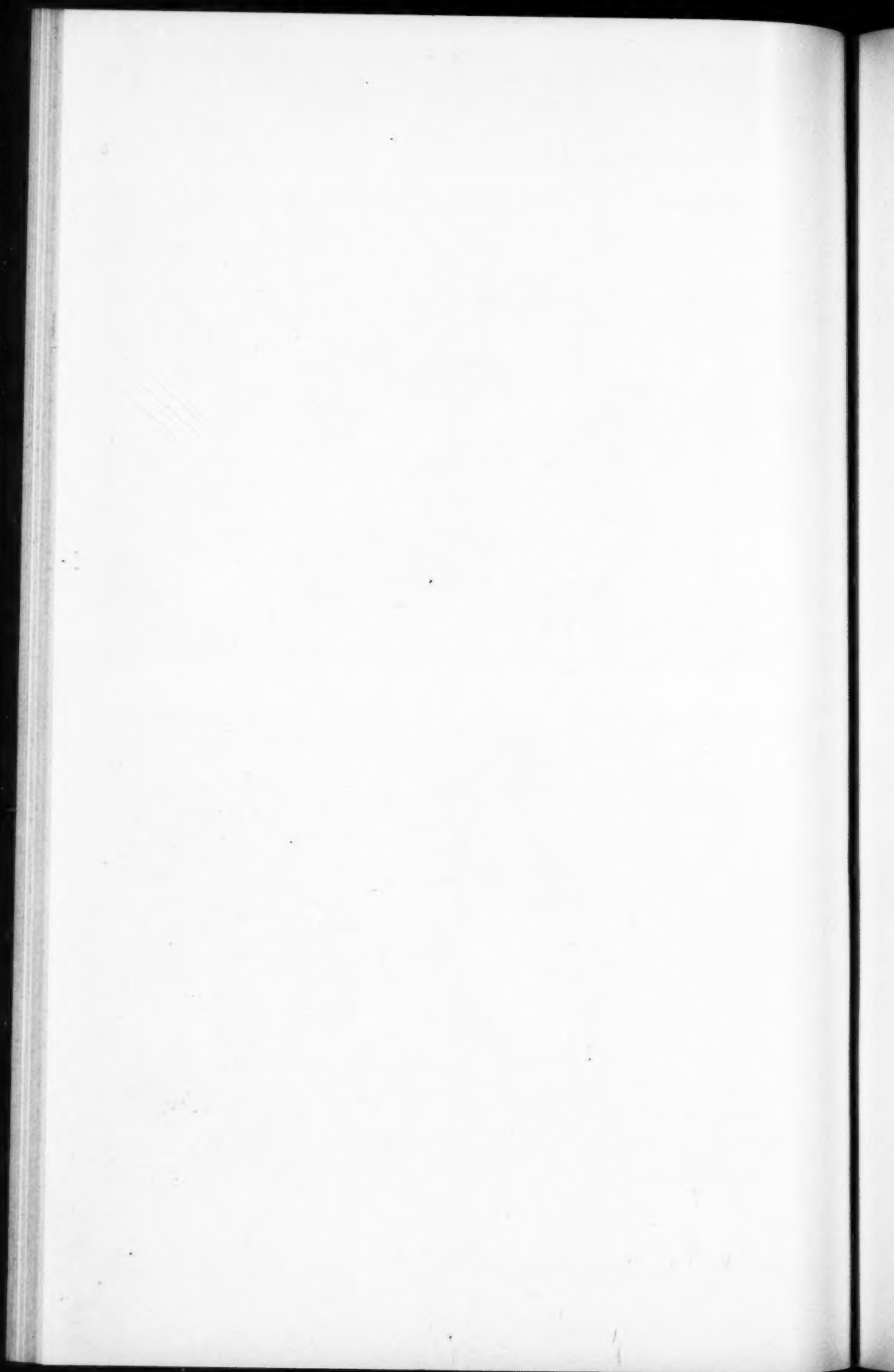
Fig. 2. From the co-type specimen, *Maximowicz*, in the Gray Herbarium of Harvard University.

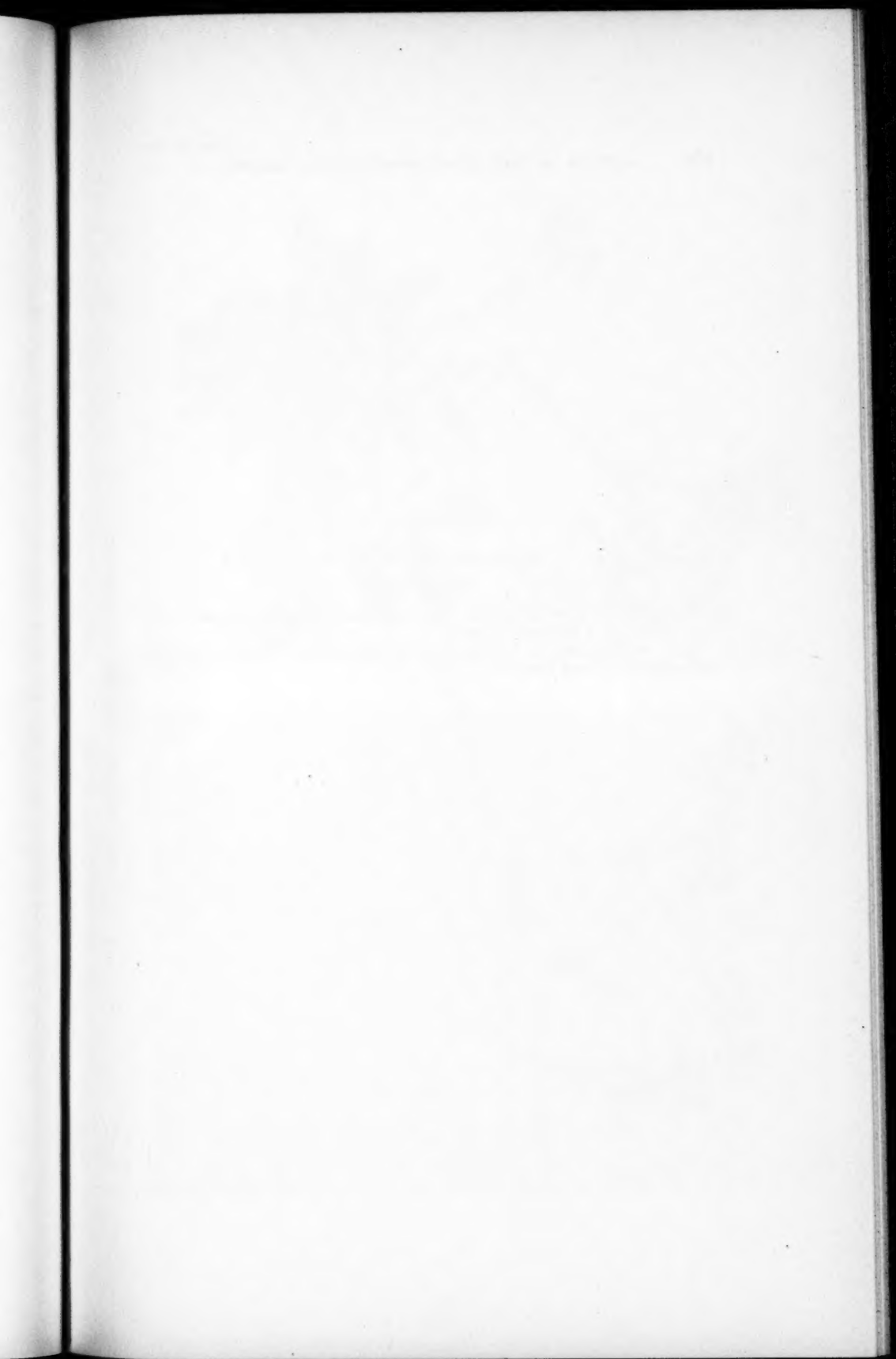


MATHIAS—STUDIES IN UMBELLIFERAE

1

2



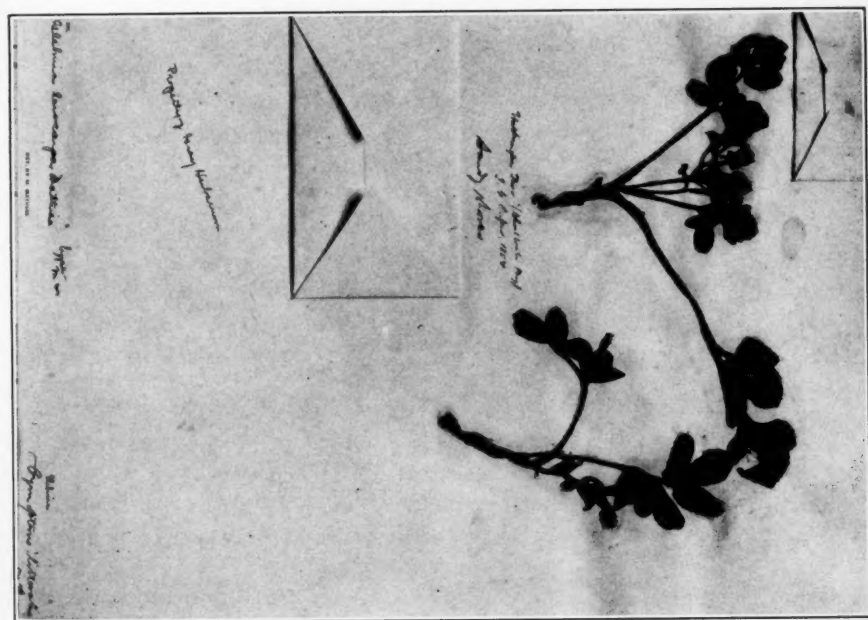
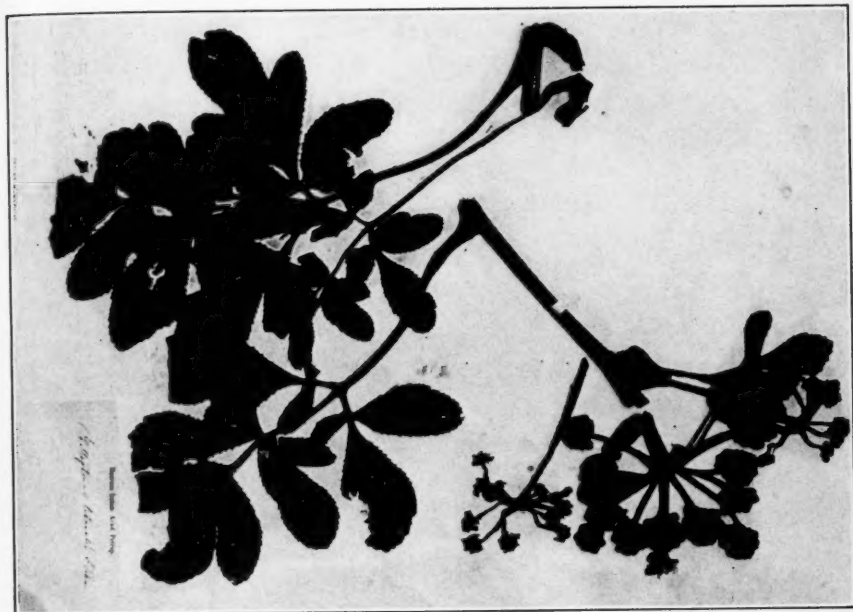


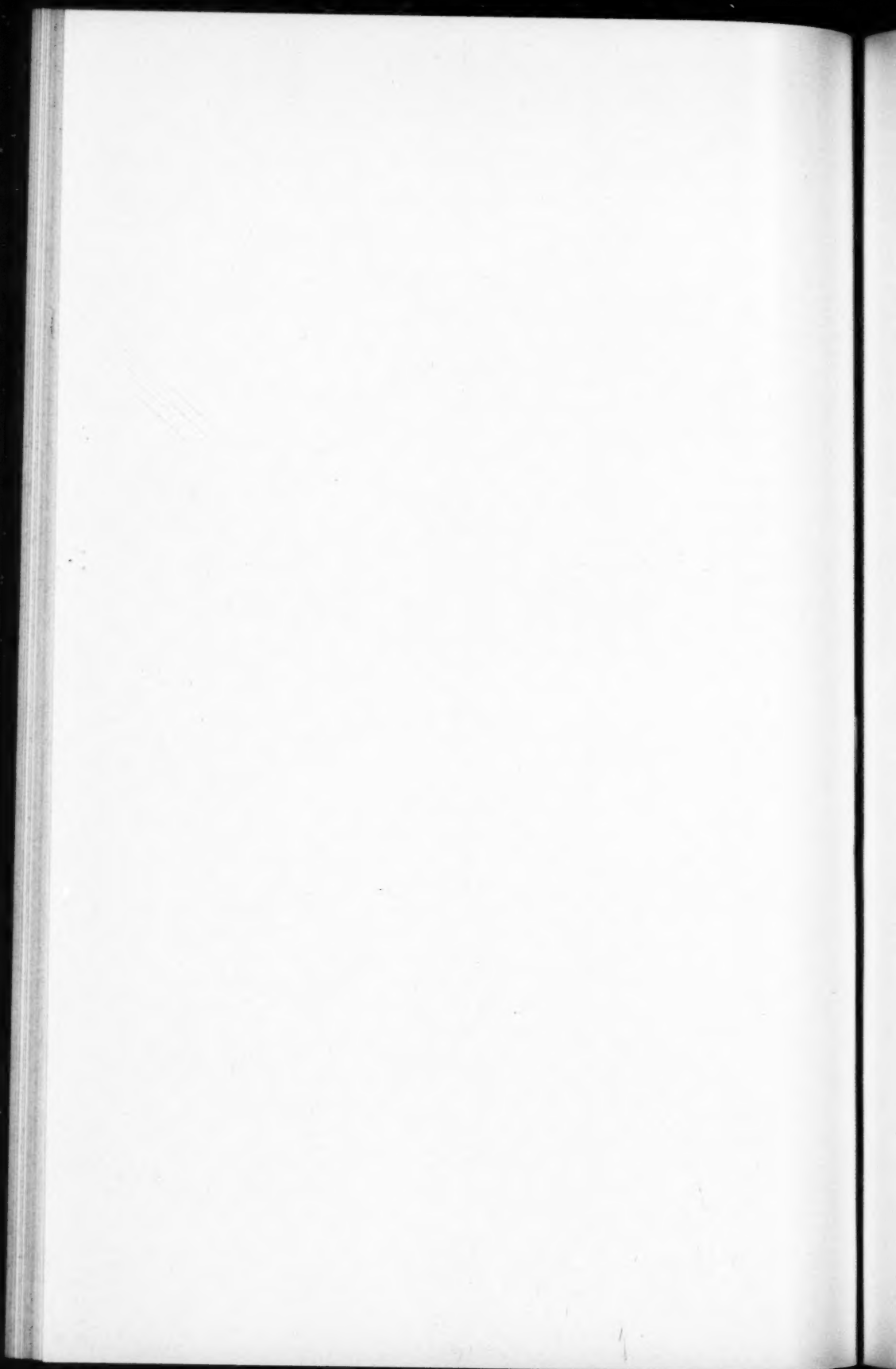
EXPLANATION OF PLATE

PLATE 19

Fig. 1. *Glehnia littoralis* Schmidt, from a specimen collected by *Schmidt*, in the Gray Herbarium of Harvard University.

Fig. 2. *Glehnia leiocarpa* Mathias, from the type specimen, *Cooper*, in the Gray Herbarium of Harvard University.





CONCERNING THE STATUS OF THE GENUS LATERNEA

DAVID H. LINDER

*Mycologist to the Missouri Botanical Garden
Instructor in the Henry Shaw School of Botany of Washington University*

While in Cuba during the summer of 1924, the writer collected a member of the Clathreae which was subsequently determined in Saccardo ('88) as *Clathrus triscapus* (Turpin) Fr.

In going over the literature concerning the simple columnar species of *Clathrus*, it was observed that there were few statements as to the manner in which the gleba is borne. Examination of the figures accompanying the original descriptions led the writer to the conclusion that the majority of these simple species carry the gleba in the same fashion as do the more complex ones in which the columns anastomose to form a latticed sphere. In these latter forms, exemplified by *Clathrus cancellatus* and *C. crispus*, the gleba is closely applied to the inside of the columns or receptacles. However, in the genus *Laternea*, of which *L. triscapa* is the original species, the columns, strictly speaking, are stipes united above, and these subtend an angular body, subovate in outline, the "lanterne" of Turpin (1822).

A comparison of *C. columnatus*, *C. crispus*, and *C. cancellatus* brings out the fact that except for the gross morphological differences, the structure of the simple columnar and the more complex latticed species is essentially the same; that is, the columns may be relatively rough or even smooth on the outside, but on the inside they are always rough and pitted (pl. 20, figs. 3-6). It is to this pitted inside surface of the columns that the gleba is applied. Studies of preserved young material of *Clathrus columnatus* and observations of the other two species in the field at all stages of development amply support this view. There is certainly no evidence that, at the time of rupture of the volva, any definite receptacle other than the column is present.

Aside from the fact that the gleba of *Laternea triscapa* is strictly confined to the angled, subovate, specialized receptacle pendant from the junction of the apices of the columns, it differs from

C. columnatus and other similar members of that genus by being proportionately taller and more slender. In addition, the columns are less angular and the surfaces are smooth, both on the inside and outside (pl. 20, fig. 1, 2).

With the above distinction in mind, it becomes quite evident that Turpin (1822) was thoroughly justified in creating the genus *Laternea* for that form which bears the gleba in the manner mentioned. Accepting this view, then *Laternea triscapa* becomes the only representative of the genus and *Laternea columnata*, *L. pusilla*, *L. rhacoides*, *L. Spegazzini*, *L. angolensis*, and possibly *L. bicolumnata*, considered as belonging to the genus by Lloyd ('09), should be excluded and treated as members of the genus *Clathrus*, following the treatment by Fischer ('86). Certainly this is a more natural grouping, especially since *Clathrus columnatus* tends towards the more complex type represented by *C. cancellatus*. The latter species may at times be columnar below and only show anastomosis of the receptacle above, while in the former, as is shown in pl. 20, fig. 6, there is a tendency for the columns to divide to produce four or even five. If, however, it is deemed more convenient to separate the simple columnar members from the genus *Clathrus*, then the genus *Colonnaria* Rafinesque (1808), on the basis of priority, should be restored, and *Clathrus* of Micheli (1729) should be reserved for those forms with anastomosed receptacles.

In view of the former uncertainty concerning *Laternea*, it seems advisable, while restoring it to its original status, to re-describe the genus, and also the species as follows:

Laternea Turpin: Columns slender, smooth, usually three, subtending from the junction of the apices an angular, subovate receptacle to which the gleba is restricted.

Laternea triscapa Turpin: columns 3, "capucine buff"¹ at base, becoming "cadmium orange" above; smooth on inner and outer surfaces, 5-6.2 cm. long, 4-5 × 6 mm. in diameter, united above; receptacle pendant, "nopal red," angled, subovate in outline, 10 × 13 mm.; gleba deep olive; volva white, 15 × 20 mm.

In sugar cane field at edge of woods, Soledad, Cuba. Sep-

¹ Ridgway, R. Color standards and nomenclature. Washington, D. C., 1912.

tember, 1924, *Linder* (in Farlow Herb. at Harvard Univ. and writer's herbarium).

In conclusion, the writer wishes to express his indebtedness to Prof. William H. Weston, Jr. for the loan of the preserved material of *Clathrus columnatus* Bosc.

BIBLIOGRAPHY

- Fischer, E. ('86). Versuch einer systematischen Uebersicht über die bisher bekannten Phalloideen. Königl. Bot. Gart. u. Mus. Berlin, Jahrb. 4: 1-92. *pl. 1.* 1886.
- Lloyd, C. G. ('09). Synopsis of the known Phalloids. pp. 48-65. *f. 59-64.* Cincinnati, 1909.
- Michelius, P. A. (1729). Nova plantarum genera iuxta Tournefortii methodum disposita. p. 214. *pl. 83.* Florence, 1729.
- Rafinesque, C. S. (1808). Prospectus of Mr. Rafinesque Schmalz's two intended works on North American botany; the first on the new genera and species of plants discovered by himself and the second on the natural history of the funguses, or mushroom-tribe of America. N. Y. Med. Repository, 2nd hexade 5 (11): 355. 1808.
- Saccardo, P. A. ('88). Sylloge Fungorum 7: 18-21. 1888.
- Turpin, P. J. F. (1822). Dict. d. Sci. Nat. 25: 248. 1822.

EXPLANATION OF PLATE

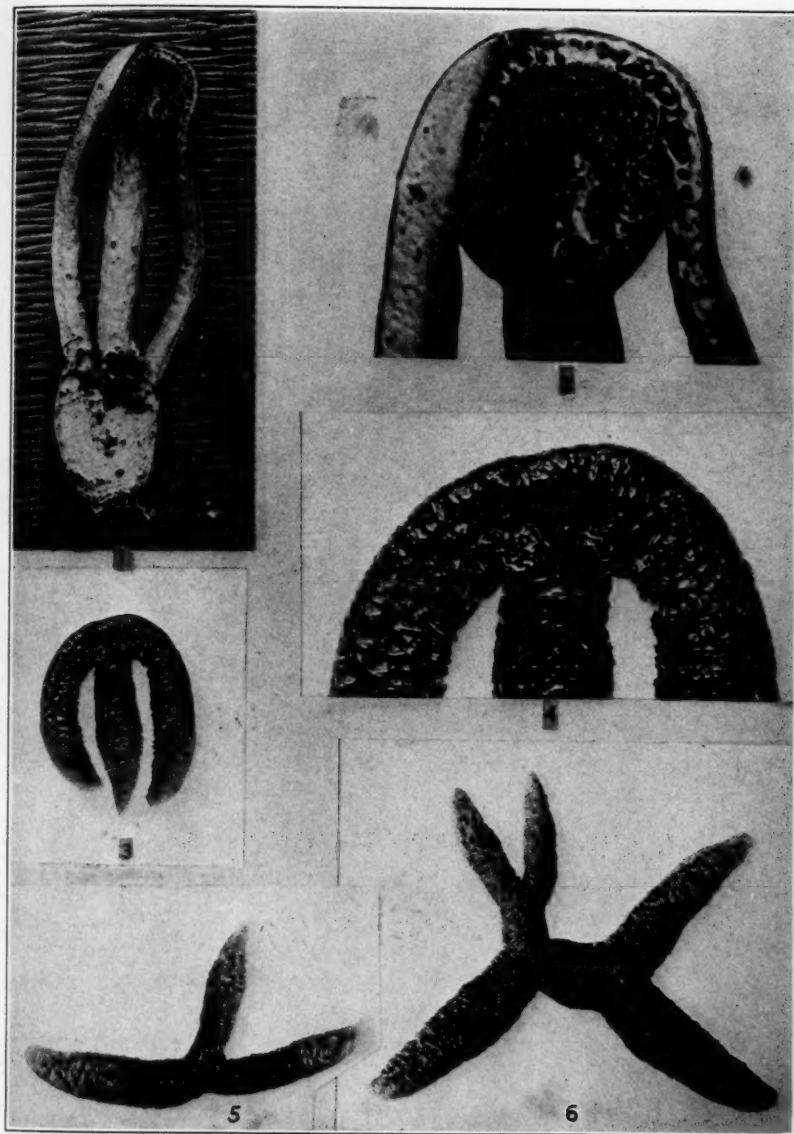
PLATE 20

Fig. 1. *Laternea triscapa*, showing the suspended, specialized receptacle bearing the gleba, and the smooth surfaces of the columns. Photograph of freshly collected specimen. Natural size.

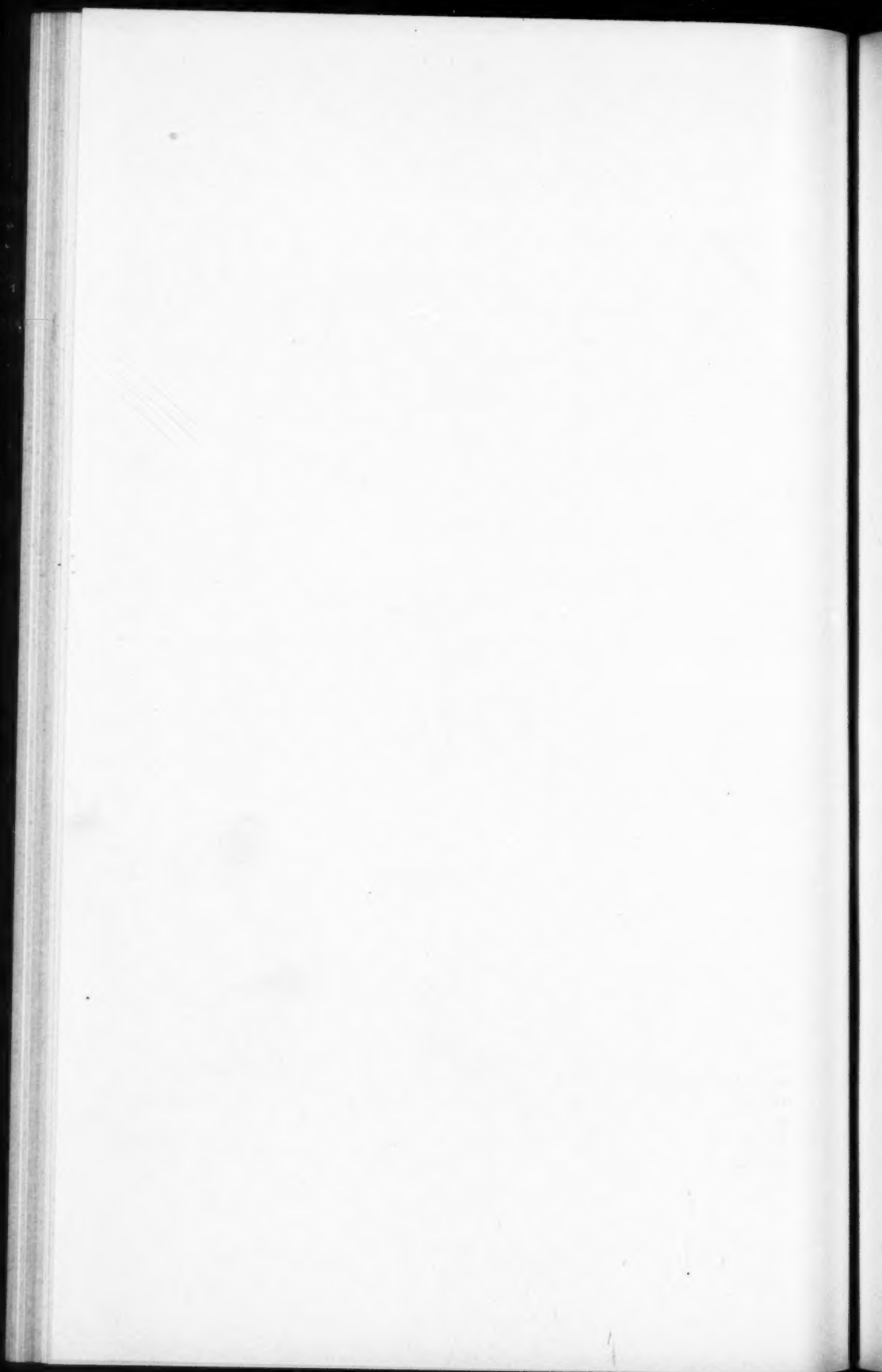
Fig. 2. *Laternea triscapa*. Receptacle and upper part of columns enlarged three times to show the definite development of tissue to form the receptacle.

Fig. 3. *Clathrus columnatus*, showing the relatively smooth outer surface and the rough, pitted inner surface. The gleba may be seen still adhering to the inner surface at the junction of the columns or receptacles. From preserved material collected in Porto Rico by P. V. Siggers. Natural size.

Fig. 4-6. *Clathrus columnatus*. Fig. 4 shows the upper portion of the receptacle enlarged three times. There is no evidence of any tissue that may be considered comparable to that found in the receptacle of *L. triscapa*, the gleba being applied to the inner pitted surface. Figures 5 and 6 show the receptacles spread out and viewed from below; the latter figure illustrates the division of one of the columns to form a 5-columnar receptacle. $\frac{4}{5}$ natural size.



LINDER—THE GENUS *LATERNEA*



SOURCES OF ENERGY FOR AZOTOBACTER, WITH SPECIAL REFERENCE TO FATTY ACIDS¹

P. L. GAINES

*Soil Biologist, Kansas Agricultural Experiment Station
Formerly Teaching Fellow in the Henry Shaw School of Botany of Washington University*

INTRODUCTION

The thermo-chemical phenomena involved in the fixation of free nitrogen by various micro-organisms are not well understood. It has been assumed that the fixation process is endothermic in nature and that the necessary energy is, in the case of the *Azotobacter* group of organisms, derived from the oxidation of organic compounds, principally of a carbohydrate, acid, or alcohol nature.

Regardless of whether the initial process through which nitrogen is brought into combination is exo- or endo-thermic, no one has been able to establish definitely a measurable fixation of nitrogen by *Azotobacter*, or any other nitrogen-fixing group of organisms, in the complete absence of some form of organic matter. Furthermore, growth and nitrogen fixation have been found to run more or less parallel with the quantity and nature of the organic material available, provided the material is non-nitrogenous in nature. It may be assumed safely, therefore, that an organic food material of some kind is essential in the metabolism of this group of organisms. This being true, it would seem highly desirable, both from a theoretical and practical standpoint, to secure as much information as possible relative

¹ An investigation carried out in part at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University and in part in the Research Laboratory of Soil Biology of the Kansas Agricultural Experiment Station, and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

to the different organic food substances suitable for these organisms and also the relative efficiency with which different compounds can be used.

As an aid in the solution of some of the more complicated thermo-chemical questions involved it seemed desirable to ascertain, if possible, whether any quantitative relationship existed between the potential energy content of the organic material utilized, on the one hand, and the quantity of growth and nitrogen fixed, on the other. It was with the hope of securing information along these lines that this work was undertaken.

More specifically this investigation has been concerned with seeking an answer to the following questions: (1) Is there any difference in the relative availability of the lower fatty acids as a source of carbon, or organic food substance, for *Azotobacter*? (2) If *Azotobacter* exhibits differences in ability to utilize different fatty acids, can such differences be associated with the structure, size, or energy content of the molecule?

The following criteria were used in judging the ability of *Azotobacter* to utilize the various acids: (a) the variation in visible growth; (b) the disappearance of the acid; (c) the fixation of nitrogen; (d) changes in the hydrogen-ion concentration of the medium.

The lower fatty acids were selected for study because they compose a series of compounds exhibiting many characteristics in common, yet in the series the molecule increases in definite increments from fairly simple to fairly complex. Furthermore, the heat of combustion of these compounds increases directly as the molecular weight increases. By including the iso compounds, two molecules varying in structure but identical in composition and energy content could be compared. An additional desirable characteristic possessed by this series of compounds is that they are found as free acids or as constituents of fats in nature, and some of them are already known to serve as organic food for *Azotobacter*. One other characteristic essential in a series of compounds suitable for a study of this nature is susceptibility to fairly easy quantitative analysis. Not many series of organic compounds of which the members possess the

desirable characteristics enumerated above can be found, and possibly there is no other series in which as many members may be used so advantageously. Unfortunately, lack of solubility prevents any quantitative use of members of this series above six carbon atoms, but even then there would seem to be enough members of the series that can be used to give valuable information if carefully studied.

METHODS

General procedure—The general procedure has been to prepare, in one batch, as carefully as possible, all the medium necessary for a single experiment. Measured quantities of this were placed in the culture flasks which were then stoppered with cotton and sterilized in the autoclave. After sterilization the flasks were inoculated as uniformly as possible with a heavy suspension of organisms washed from the surface of mannitol-soil-extract agar upon which active vigorous growth was taking place. No old or apparently non-vigorous growing culture was used as an inoculum. After varying periods of incubation the cultures were removed from the incubator and the qualitative and quantitative analyses were made as indicated.

Where supplementary aeration was resorted to, an inlet tube of glass, containing five to seven small openings near the end, was inserted in a rubber stopper in such a way that when the stopper was tight in the culture flask the end of the tube almost reached the bottom of the flask. The stopper was also provided with an outlet tube to be connected to a vacuum system. The stopper and connecting tubes were sterilized separately and inserted after inoculation. Cotton was forced into the ends of the connecting tubes as a precaution against possible contamination. In the aerated experiments 300-cc. Pyrex Erlenmeyer flasks were used as culture containers, and all those in any one experiment were connected in series so that the same quantity of aeration was provided for all. While incubating, a vigorous bubbling of air through the media was continuously maintained. Before entering the first culture flask the air was washed through flasks arranged as mentioned above, of acid, alkali, and water. Despite special precautions to prevent contamination there was one type of foreign organism difficult to keep out.

Where no special aeration was provided the cultures consisted of 50 cc. of media in 300-cc. flasks; 100 cc. media in 750-cc. flasks; or 200 cc. media in 1000-cc. Pyrex Erlenmeyer flasks. These quantities of media in the flasks indicated always exhibited a large surface area compared to the depth, and while aeration was certainly not as vigorous as where air was drawn through the culture, nevertheless it was ample for very rapid growth. Growth at the bottom of the culture was frequently observed before it made its appearance on the surface, indicating aeration throughout the culture. These cultures were left stationary except when being handled for examination, and even then care was taken not to shake so vigorously as to break up any film that might be forming on the surface.

Medium—Unless otherwise stated the medium used in the various experiments had the following composition, and its suitability is evidenced by the very rapid growth that took place when the organic material was assimilable:

K_2HPO_4	2.50 gms.
$MgSO_4$20 gm.
$NaCl$20 gm.
$CaCl_2$05 gm.
$FeCl_3$ (10 per cent sol.).....	1. drop
Organic material.....	1 per cent
Distilled water.....	1000 cc.

In some of the preliminary experiments only 0.50 gm. of K_2HPO_4 was used, but it was observed that when such a small quantity of phosphate was added the hydrogen-ion concentration sometimes changed so rapidly and markedly that growth was very soon inhibited by the increase of hydroxyl-ions. A rapid increase in hydroxyl-ion concentration was always observed when large quantities of a metallic salt of an organic acid were metabolized, and probably arose from the formation of an hydroxide by the metallic-ions set free when the acid radicle was assimilated by the organisms.

Even with 2.5 gms. phosphate and an excess of $CaCO_3$, the buffering effect was barely sufficient to permit of complete oxidation of 1.0 per cent acid. In fact, in some instances there is evidence to indicate that the high alkalinity accompanying vigorous oxidation not only prevented further activity, as is

evidenced by the failure of the organisms in certain cultures to oxidize all the acid even where abundant growth occurred, but actually resulted in the death of most or all of the organisms present. In case of some of the less soluble acids the quantity added was only 0.5 per cent.

When the organic material was fatty acid it was, in all except a few preliminary experiments, added to the entire volume of distilled water. An excess of CaCO_3 was then added, and the material boiled until the reaction became neutral to brom-thymol-blue, indicating complete transformation into the calcium salt, after which it was filtered. This procedure was resorted to in order to hasten the completion of the reaction between the weakly dissociated acid and calcium carbonate. The other salts were then added, and if any change in the reaction took place it was again adjusted to neutrality by the addition of sodium hydroxide or sulphuric acid as needed. The medium was then measured into the culture flasks, care being taken to keep it well agitated in order to secure an equal distribution of the precipitate, a small quantity of CaCO_3 added, the flask plugged with cotton and sterilized in the autoclave at ten pounds pressure. The final reaction of medium prepared as indicated was never far from pH 7.0.

Obviously, one could not depend upon the original weight of any organic material subjected to the manipulations described in the preceding paragraph as indicating the final concentration. It was necessary, therefore, to prepare controls and make quantitative analyses of the final concentration of the organic materials in all cases.

The agar used for maintaining stock cultures, for testing the purity of cultures, and for the preparation of the inoculum was a soil-extract-mannitol agar prepared as follows: One thousand gms. fertile garden soil were added to 1000 cc. distilled water and subjected to fifteen pounds pressure in the autoclave for thirty minutes, after which CaCO_3 was added and the mixture filtered. The clear filtrate was made up to 1000 cc. with distilled water. To 900 cc. distilled water was added 100 cc. soil extract, 0.5 gm. K_2HPO_4 , 10 gms. mannitol, and 15 gms. agar agar. After heating in the autoclave to bring the agar into

solution, and while still hot, phenolphthalein and sufficient sodium hydroxide were added to give a distinct pink color.

Cultures—The following include all cultures employed in any experiment, together with their origin. They were selected from among more than one hundred available cultures of *Azotobacter*. Those strains that were used to any appreciable extent were selected primarily because of their vigorous growing and nitrogen-fixing ability. Cultures Nos. 3a and 3b were strains of *Azotobacter chroococcum* secured from S. A. Waksman, of New Brunswick, N. J. Cultures Nos. 4, 5a, 5b, 6, 7, 8, 57, 58, 59, 60, 62, and 66 were isolated from different Colorado soils in the laboratory of W. G. Sackett, Fort Collins, Colo. Culture No. 94 was a strain of *Azotobacter vinelandii* from the Bureau of Plant Industry, U. S. Dept. Agr., Washington, D. C. Culture No. II was received from W. Omeliansky, Leningrad, Russia. Culture No. 218, a strain of *Azotobacter chroococcum* marked "K," was received from Chr. Barthel, Stockholm, Sweden. Cultures "C" and "R" were received from the Rothamsted Experiment Station, England. "C" came originally from a single cell strain of H. R. Christensen's and "R" was isolated from soil. Cultures Nos. 178, 187, 188, 194, and 165 were all isolated in this laboratory from the following soils, respectively: No. 178, Gloucester loam from Minnesota; No. 187, field soil from New York; No. 188, cotton and sugar cane soil, Virgin Islands; No. 194, soil from V. L. Winogradsky, Paris, France; No. 165, irrigated potato field soil from Wyoming. The only unidentified strain used to any appreciable extent was No. 62. This culture possessed the characteristics of *Azotobacter chroococcum* in that it grew abundantly as grayish-white opaque, distinct colonies, soon turning brown and eventually black with a more or less wrinkled dry surface.

Before any culture was used in any experiment it was carefully tested for purity by repeated streaking and re-isolation from individual, microscopically examined colonies, until assured of the presence of only one type of organism. Furthermore, after incubation most cultures were again examined for purity and if evidence of contamination was present it has been so recorded.

Inoculum.—The inoculum was prepared by streaking the

entire surface of a Kolle flask of soil-extract-mannitol agar with the desired culture, incubating 48–96 hours, or until the entire surface was covered with a uniform thick growth, and suspending this growth in 25–50 cc. of sterile water. This gave a suspension of such density as to be practically opaque in a depth of only half an inch. One or two per cent of this was used as the inoculum, thus insuring a very heavy inoculation.

Incubation.—All cultures were incubated either at summer room temperature or in an incubator at 28–32° C. Room temperature was quite favorable in the summer but during the winter the temperature dropped too low at night for active growth. Most of the aerated experiments were run at room temperature, while all non-aerated experiments were incubated at 28–32° C.

CHEMICAL METHODS

Dextrose.—Quantitative dextrose determinations were made by the Shaffer-Hartmann ('21) iodometric method. Where a heavy growth of *Azotobacter* had taken place the slime-like material present was precipitated by adding 1.0 per cent of a mixture of 2.5 gms. phosphotungstic acid and 5.0 gms. H_2SO_4 before sugar determinations were made. Preliminary experiments proved that sugar could be recovered quantitatively when added to a culture and treated by this method.

Total nitrogen.—The Gunning modification of the Kjeldahl method was employed for total nitrogen. If the culture consisted of only 50 cc. of medium the entire volume was utilized, otherwise after making the culture up to the original volume 25-cc., or more frequently 50-cc., duplicated samples were run. A small piece of copper wire, 7 gms. of anhydrous sodium sulphate, and 35 cc. H_2SO_4 were added and digestion continued for one hour after the solution became clear (see Latshaw, '16). Table I indicates the degree of accuracy with which duplicate determinations checked. In some of the experiments where aeration was employed the data for nitrogen determinations did not seem conclusive, and it has been thought best to leave them out entirely. No significance is attached to an increase in nitrogen of less than 0.5 mgs. per 100 cc. of medium, and all the data recorded in the tables are based upon 100 cc.

TABLE I

ACCURACY WITH WHICH TOTAL NITROGEN DETERMINATIONS CHECKED

Sample No.	Mgs. nitrogen recovered from		
	Peptone solution	Peptone solution	Azotobacter culture
1	9.58	9.61	2.21
2	9.52	9.52	2.34
3	9.61	9.52	2.14
4	9.58	9.58	2.21
5	9.52	9.52	2.08
6	9.52	9.58	2.27
Average	9.55	9.55	2.25

Volatile acid.—Practically all the acids used were Eastman Kodak Co. products. Quantitative determinations have been made by distilling 100 cc. from a total volume of 110 cc. Pyrex Erlenmeyer flasks of 300 cc. capacity connected to Liebig condensers and surrounded by an asbestos shield were used as distillation flasks. These were heated by an electric hot plate, and it required 30–45 minutes, depending upon the acid, to distil over 100 cc. Titrations were made in increments of 20 cc. unless it had previously been noted that practically all acid had disappeared, in which case the entire 100 cc. were titrated at one time. This fractional titration was employed in order to enable the plotting of the titration curves to detect the transformation of a higher into a lower acid. Phenolphthalein was employed as an indicator, and care was exercised that all vessels and wash water were neutralized before being used.

Figures 1 and 2 are given to show the relative titration curves of the different normal acids and also to show that there was no indication of an acid with a higher molecular weight being transformed into one of lower molecular weight. The curves for the standard acid solution and for the cultures in which abundant growth had taken place coincide as well as would curves from two different batches of acid. The culture distillation curves are from cultures in which approximately half the original acid had disappeared. Curves for iso compounds would show the same thing. These curves are plotted on a basis of the per cent of the total recovered that came over in each 20-cc. fraction, when 100 cc. were distilled from a total volume of 110 cc.

Calculations of the quantity of acid present were based upon quantitative distillations of carefully standardized acids distilled from pure water to which a small quantity of sulphuric acid had also been added. The data in table II show the per cent

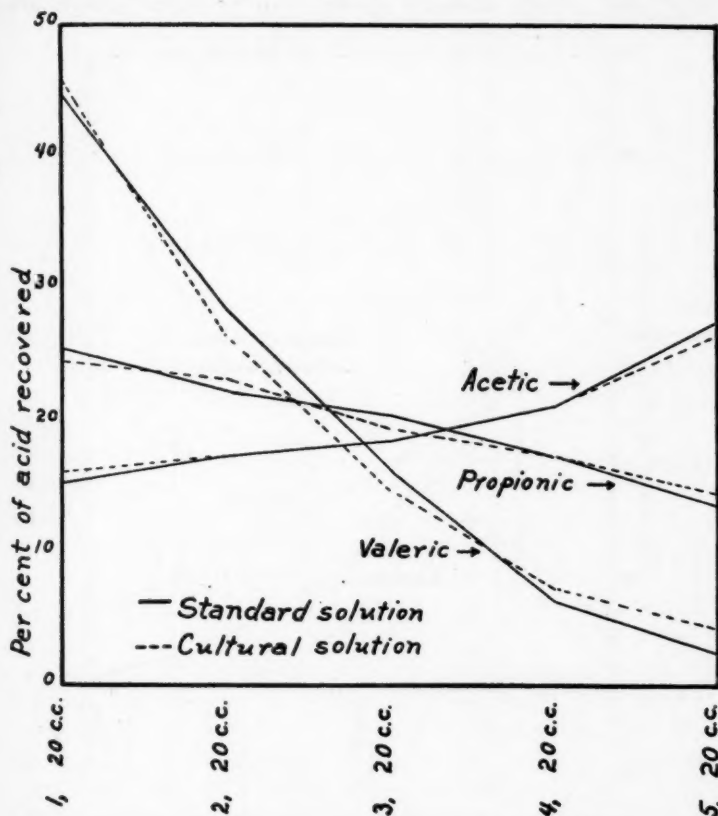


Fig. 1. Distillation curves for volatile acids from standard solutions and from cultures in which approximately half of the acid had been metabolized.

of the total recovered from the different acids when varying quantities up to 100 cc. of the total 110 cc. had been distilled. Data recorded in table III show the degree of accuracy with which triplicate determinations checked. The figures in table

iv indicate that the presence of the other constituents of the culture medium did not interfere with the recovery of the volatile acid. The quantity of medium distilled was, unless indicated to the contrary, 25 cc., and duplicate or triplicate samples were always run. Only averages of the two or three checks are

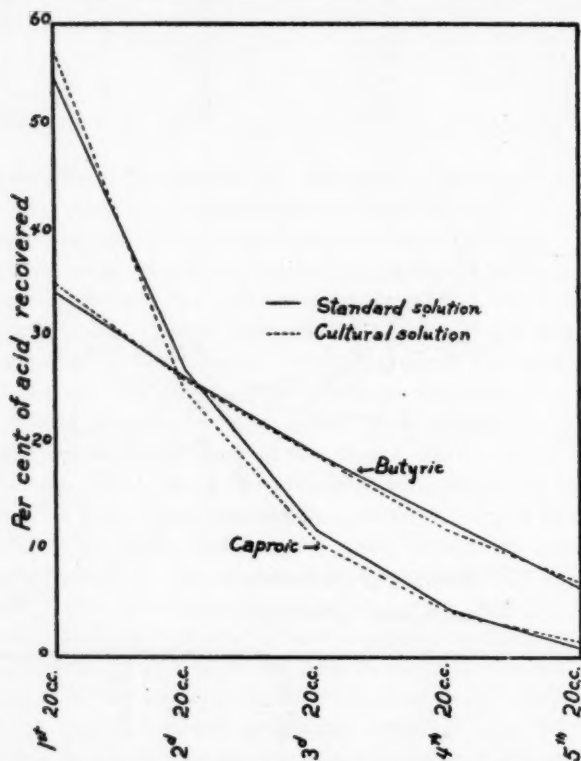


Fig. 2. Distillation curves for volatile acids from standard solutions and from cultures in which approximately half of the acid had been metabolized.

recorded. When distilled from the culture medium the volatile acid was freed from calcium by adding an excess of sulphuric acid. The CO_2 thereby liberated was removed by aerating vigorously for thirty minutes.

In the aerated cultures, run for the longer periods of time,

TABLE IV

RECOVERY OF ACIDS FROM PURE WATER SOLUTION AND FROM AN
AZOTOBACTER CULTURE MEDIUM; EXPRESSED IN EQUIVALENT
QUANTITIES OF N/20 NaOH

Cc. distilled	Butyric		Valeric		Caproic		Propionic	
	H ₂ O	Medium	H ₂ O	Medium	H ₂ O	Medium	H ₂ O	Medium
20	8.40	8.45	11.40	11.60	9.25	9.45	5.95	5.95
40	14.70	14.95	17.60	17.90	13.80	14.00	11.60	11.95
60	19.50	19.80	20.85	20.95	15.82	16.00	16.60	16.65
80	22.80	23.10	22.10	22.15	16.55	16.70	21.10	21.20
100	24.65	24.80	22.56	22.50	16.75	16.85	25.00	25.10
Total present	25.25	25.45	22.80	23.00	16.90	17.05	26.90	27.10
Per cent recovered	97.60	97.50	98.90	98.00	99.10	98.80	92.90	92.60

there were indications in some instances that slight losses of acid occurred. This is not surprising, though, in view of the fact that despite four wash-bottles, designed to remove any acids and bases that might be present in the laboratory air as well as saturate the air with moisture, there were sometimes rather large losses in the volume of culture medium. Such losses were no doubt principally due to evaporation but probably in a less degree to the mechanical removal of moisture due to the bursting of so many bubbles. Owing to such discrepancies it has been difficult to evaluate accurately the utilization of acid by the growing cultures in certain instances. We have therefore recorded as questionable such losses in the aerated experiments unless they obviously exceeded such losses where observations were possible.

Qualitative reaction.—It has been thought best to check roughly (time would not permit accurate determination) the hydrogen-ion concentration of the cultures in order to determine if the reaction were suitable for growth. For this purpose use has been made of brom-cresol-purple, brom-thymol-blue, phenol-red, cresol-red, phenolphthalein, and thymol-blue. Since the medium was only tested roughly as to whether it was acid, alkaline, or neutral to those indicators within whose range its reaction lay, the figures recorded in the various tables are merely approximate. Only in those instances where the medium was found to be alkaline to thymol-blue and is recorded as 9.0 + has there been any indication that the reaction was unfavorable. In all

probability a medium alkaline to thymol-blue has an unfavorable, if not toxic, effect upon *Azotobacter* (see Johnson and Lipman, '22).

PRELIMINARY EXPERIMENTS

A large number of experiments of a preliminary nature have been performed. In fact, before any method, or step in a method, or culture was adopted for experimental use it was carefully tried to see that it would work satisfactorily. Among these preliminary experiments there are, aside from those already mentioned, a number that seem to be of sufficient significance to record.

Experiments reported by Hunter ('23) indicated that by drawing a current of air through the medium, growth of *Azotobacter* and fixation of nitrogen could be greatly stimulated. Since the use of any method that would hasten growth seemed desirable, in view of the slow assimilation of certain of the fatty acids reported by Mockeridge ('15), it was thought that Hunter's method might be used advantageously. Therefore, an experiment was designed not only to confirm Hunter's results but at the same time to determine whether varying the rate of aeration would influence quantitatively the consumption of the organic food and the fixation of nitrogen. The results of such an experiment are reported in table v.

No method of measuring quantitatively the rate of flow of air through the medium was available; however, in the samples subjected to "slow" aeration a slow continuous flow of bubbles, perhaps one a second, was maintained. In the "medium" aerated cultures the air was drawn through at least ten times as rapidly, while the cultures subjected to "rapid" aeration probably received ten times as much air as the "medium" aerated cultures.

The data show very definitely that increasing the rate of aeration increases both the rate of dextrose consumption and nitrogen fixation. There is some indication that the dextrose may possibly be utilized somewhat more efficiently with limited aeration, the average nitrogen-dextrose ratio being 1:79 for the "slow" aerated samples, whereas the corresponding ratio for the other samples was 1:115 and 1:113 respectively.

TABLE V
EFFECT OF AERATION UPON THE UTILIZATION OF DEXTROSE AND THE FIXATION OF NITROGEN BY CULTURE NO. 3A.
INCUBATION 4 DAYS

Flask No.	Aeration "slow"			Aeration "medium"			Aeration "rapid"		
	Mgs. dextrose consumed	Mgs. nitrogen fixed	Mgs. dextrose per mg. nitrogen fixed	Mgs. dextrose consumed	Mgs. nitrogen fixed	Mgs. dextrose per mg. nitrogen fixed	Mgs. dextrose consumed	Mgs. nitrogen fixed	Mgs. dextrose per mg. nitrogen fixed
1	104	1.38	75	622	5.37	116	954	8.82	108
2	108	1.24	87	693	5.64	123	954	8.41	113
3	108	1.52	71	606	4.82	126	954	8.27	115
4	96	1.38	70	690	6.85	101	954	8.27	115
5	112	1.24	90	412	3.45	119	954	8.41	113
Average	105	1.35	79	605	5.25	115	954	8.44	113

In this connection it might be well to comment, in passing, upon the use of the term "dextrose-nitrogen ratio." Certain investigators in speaking of this relationship have made use of the term "carbon-nitrogen ratio." This term, it is believed, does not accurately express the relationship. If it is a question of the organism securing or setting free a certain quantity of energy per unit of carbon, as is usually considered, obviously the expression C : N ratio is incorrect in that the energy freed per unit of carbon depends upon the other elements combined with the carbon as well as upon the carbon. For example, caproic acid contains approximately twice as much energy per gram of material and one and one-fourth times as much energy per gram of carbon as does dextrose, both being six carbon atom compounds. It would seem, therefore, that it would be much more logical to express this relationship upon an energy or molecular basis.

In an effort to secure a desirable culture with which to carry out the more extensive investigations recorded in the next part of this paper, the experiments reported in tables VI and VII were designed. All the cultures available at that time were included in these tests.

TABLE VI
VARIATION IN UTILIZATION OF DEXTROSE AND FIXATION OF
NITROGEN BY DIFFERENT CULTURES OF AZOTOBACTER

Culture No.	Incubation 2 days			Incubation 5 days		
	Mgs. dextrose consumed	Mgs. nitrogen fixed	Mgs. dextrose per mg. nitrogen fixed	Mgs. dextrose consumed	Mgs. nitrogen fixed	Mgs. dextrose per mg. nitrogen fixed
3a	169	2.20	77	620	4.96	125
3b	197	2.48	79	674	6.06	111
4	171	1.38	124	522	1.10	474
5a	179	2.06	82	679	3.58	189
5b	169	2.34	72	729	3.58	203
6	247	2.34	106	692	3.04	227
7	157	.82	192	250	.82	610
8	148	1.38	107	368	1.66	222

These data indicate that culture No. 3a was approximately as efficient in fixing nitrogen as any other. At the same time

TABLE VII

UTILIZATION OF DEXTROSE AND NORMAL BUTYRIC ACID* AND FIXATION OF NITROGEN BY DIFFERENT CULTURES OF AZOTOBACTER

Culture No.	Mgs. dextrose utilized	Mgs. nitrogen fixed	Mgs. dextrose used per mg. nitrogen fixed	Mgs. butyric acid utilized	Mgs. nitrogen fixed	Mgs. butyric acid used per mg. nitrogen fixed
3a	957	5.40	177	99	1.38	72
3b	957	4.55	210	98	1.76	55
4	84	.50	168	3	.00	—
5a	957	5.40	177	96	1.65	58
5b	957	6.20	154	99	1.24	80
6	620	3.02	208	99	1.38	72
7	84	.28	300	0	.00	—
8	957	3.44	284	99	.82	121

* Butyric acid neutralized with sodium hydroxide.

it grew abundantly, thus enabling ready detection of growth. It was also known to be a strain of *Azotobacter chroococcum*. For these reasons it was temporarily selected for further study.

These data also indicate that certain cultures (Nos. 4 and 7), to all appearance *Azotobacter*, developed very poorly in the dextrose and not at all in the butyric acid medium. When measured by the quantity of nitrogen fixed per unit of organic material used, the actively growing cultures were apparently capable of utilizing butyric acid much more effectively than dextrose. In this particular experiment the increased effectiveness with which butyric acid was used might have been due to the relatively low per cent present, compared with dextrose. It has been frequently observed that in the presence of small quantities of organic material a higher fixation of nitrogen takes place per unit of organic material consumed. In this experiment only 0.1 per cent butyric acid was present.

Having selected culture No. 3a the next step was to test it in a preliminary way with several acids. Accordingly, the experiment recorded in table VIII was arranged. For some unknown reason this culture was only able to utilize acetic acid among those tested. Similar results were secured in other experiments.

Other cultures having been added to our collection, further qualitative tests were carried out, among which was the protocol

TABLE VIII
UTILIZATION OF VARIOUS ACIDS BY CULTURE NO. 3A

Acid	Mgs. recovered per 100 cc. culture solution					
	Controls		Incubation period in days			
	Un-aerated	Aerated	2	5	9	13
Formic	644	696	716	659	635	673
Acetic	1026	977	982	431	394	264
Propionic	1051		998	996	1002	996
Butyric	1018	1001	994	984	997	1001
Valeric	920	896	896	884	875	884
Dextrose	934	954	690	423	000	000

arranged in table ix. This experiment was also designed to gain some information relative to the effect of the cation as well as the acid radicle. The salts included were the only ones available of those particular acids at that time.

The results presented in table ix indicate very strongly that the cation is probably of as much significance in determining the availability of an acid as is the anion. None of the formates permitted growth. This has been characteristic of formic acid in all tests conducted with it and is probably due to the formation of formaldehyde. Aluminum acetate appears to be the most readily available salt of acetic acid tested, all the strains being able to assimilate it readily. Uranium acetate, on the other hand, was not assimilated by any of the cultures. Between aluminum and uranium lay calcium, ammonium, magnesium, potassium, and sodium salts, their availability being approximately in the order given.

Ammonium acetate, while apparently assimilated by all ten cultures, supported vigorous growth only in two instances. It is possible that when supplied with nitrogen the very small quantities of organic impurities finding their way into the cultures enabled the various cultures to make perceptible growth. This, though, does not seem probable. Only half the cultures were capable of making visible growth when the sodium salt was the only source of organic matter supplied, and only one out of the ten assimilated it readily. Culture No. 60 could not even metabolize dextrose readily.

This experiment, as well as others reported in this paper,

TABLE IX
UTILIZATION OF VARIOUS SALTS OF FORMIC AND ACETIC ACIDS BY DIFFERENT CULTURES OF AZOTOBACTER.
INCUBATED 48 DAYS

Culture No.	Ammonium formate	Calcium formate	Sodium formate	Ammonium acetate	Aluminum acetate	Calcium acetate	Magnesium acetate	Potassium acetate	Sodium acetate	Uranium acetate	Dextrose
3a	—*	—	—	+	+	+	+	+	+	—	+
4	—	—	—	+	+	+	+	+	+	—	+
5a	—	—	—	+	+	+	+	+	+	—	+
6	—	—	—	+	+	+	+	+	+	—	+
57	—	—	—	+	+	+	+	+	+	—	+
58a	—	—	—	+	+	+	+	+	+	—	+
59	—	—	—	+	+	+	+	+	+	—	+
60	—	—	—	+	+	+	+	+	+	—	+
62	—	—	—	+	+	+	+	+	+	—	+
66	—	—	—	+	+	+	+	+	+	—	+

* In this and all succeeding tables a minus sign indicates no growth; a question mark indicates questionable growth; while the number of plus signs indicate the relative growth observed in that particular experiment. (The same number of plus signs indicate more or less comparable growth in different experiments.)

indicates a very wide variability in the metabolism of organisms belonging to the genus *Azotobacter*. The data also emphasize the great need for more specific physiological studies of this very interesting group of organisms. It would appear utterly futile to attempt to apply the findings from the study of one strain or species to any other strain or species. Just as Löhnis and Smith ('16) have pointed out the futility of attempting to apply the morphological findings in any particular medium or at any particular time to the group as a whole, it is well to emphasize the same with regard to physiological studies.

Since culture No. 62, apparently a strain of *Azotobacter chroococcum* and hence very closely related to culture No. 3a, seemed from the above-reported experiment, as well as from a number of unrecorded tests, to possess the ability to utilize a wider variety of salts of fatty acids than any other available culture, it was selected for the more intense studies reported in the next part of this paper. Also, even though aluminum acetate was undoubtedly more readily available to some strains of *Azotobacter* than the calcium salt, the latter served equally as well for culture No. 62; and since calcium salts have found a much wider use in biological studies than aluminum it seemed desirable to use the calcium salt, thus making any results that might be secured more comparable with those reported by others. In addition, calcium salts are somewhat more easily prepared than aluminum. Calcium was therefore used as the basic element in succeeding studies.

The question of the influence of the cation should certainly receive more study, and it is hoped that such studies may be continued in the near future. The data presented in table ix are only indicative of what may be expected.

EXPERIMENTS WITH CULTURE No. 62

As previously mentioned, culture No. 62 probably belonged to the species *Azotobacter chroococcum*. Preliminary experiments indicated that it was a vigorously growing and strong nitrogen-fixing strain when supplied with a suitable form of organic material such as dextrose and certain of the lower fatty acids. The experiments conducted with this culture were all aerated.

Aeration was employed because the work of Mockeridge ('15) indicated that the rate at which certain organic materials were assimilated was extremely slow, long periods of incubation being necessary to insure appreciable utilization. Previous work had shown that the rate of growth, consumption of certain sugars, and fixation of nitrogen could be materially facilitated by drawing a current of air through the medium. It was hoped, by employing a similar method in these experiments, to shorten the time of incubation necessary to secure quantitative results of a definite character. Increased aeration unquestionably stimulated growth in many instances, but occasionally some difficulty was experienced in obtaining entirely satisfactory checking in quantitative nitrogen and volatile acid determinations following prolonged aeration, and in addition it was more difficult to maintain pure cultures. For these reasons the quantitative experiments in which *Azotobacter vinelandii* was employed were not aerated.

Utilization of formic acid.—Experiments were carried out in which formic acid was used as the sole organic constituent of the medium, but there was no indication of either growth, utilization of the acid, or fixation of nitrogen, and therefore the data are not recorded.

Utilization of acetic acid.—The data with regard to the utilization of acetic acid, recorded in table x, are quite conclusive in showing that the calcium salt of this acid is readily available to culture No. 62. Within seven days practically all the original 1.0 per cent of acid had disappeared, accompanied by abundant growth and a marked change in the reaction of the medium. In fact, it is probable that the hydroxyl-ion concentration was such as to inhibit further growth. Quite marked fixation of nitrogen was evident, but owing to an error in the method employed in the total nitrogen determinations in this experiment, the data are not recorded.

Utilization of propionic acid.—The ability of culture No. 62 to utilize readily the calcium salt of propionic acid is quite evident from the data presented in table xi. Within nine days practically all the acid had disappeared, and a marked increase in the hydroxyl-ion concentration and nitrogen content of the cultures had occurred.

TABLE X
UTILIZATION OF ACETIC ACID BY CULTURE NO. 62

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed
1	Control 0	Sterile	7.0-7.4	1059	—	Nitrogen fixation took place but owing to error in method the results were unsatisfactory.
2	Control 0	Sterile	7.0-7.4	1062	—	
3	2	Pure	7.0-7.4	831	229	
4	2	Pure	7.0-7.4	906	154	
5	4	(a)*	7.0-7.4	668	392	
6	4	(a)	7.0-7.4	654	406	
7	7	(a)	8.6-9.0	36	1024	
8	7	(a)	8.6-9.0	85	975	
9	9	Pure	9.0+	39	1021	
10	16	Contaminated	9.0+	48	1012	
11	16	Pure	9.0+	48	1012	
12	22	Contaminated	9.0+	124	936	
13	22	Pure	9.0+	91	969	

* Not tested.

TABLE XI
UTILIZATION OF PROPIONIC ACID BY CULTURE NO. 62

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed	Mgs. acid used per mg. nitrogen fixed
1	Control 0	Sterile	7.0-7.4	958	—	—	—
2	Control 0	Sterile	7.0-7.4	952	—	—	—
3	Control 18	Contaminated	8.2-8.6	17	—	—	—
4	Control 18	Sterile	7.0-7.4	974	—	—	—
5	3	Pure	7.0-7.4	829	132	1.72	76
6	5	Pure	7.0-7.4	705	256	4.74	54
7	7	Pure	7.0-7.4	626	335	3.64	92
8	9	—	9.0+	21	940	—	—
9	12	Pure	9.0+	37	924	4.74	196
10	18	Contaminated	9.0+	17	944	—	—

In this experiment control flasks Nos. 1 and 2 were not aerated, while Nos. 3 and 4 were aerated under the same conditions and for as long a time as any of the inoculated flasks. The culture adjacent to control culture No. 3 foamed badly, resulting in the contamination of No. 3 with *Azotobacter*; therefore it is not considered in the quantitative calculations. It is evident,

though, from a comparison of control flasks No. 1 and No. 2 with No. 4 that no volatilization of the propionic acid took place. Therefore the decrease in the quantity of propionic acid that occurred in the presence of pure cultures must have been due to its assimilation by the organisms.

Utilization of normal butyric acid.—The data presented in table XII show that culture No. 62 is also capable of utilizing normal butyric acid in its metabolism. Again only seven days were required for almost complete assimilation of the acid present, with corresponding decreases in the hydrogen-ion concentration. The quantities of nitrogen fixed were also marked, as was the visible growth of the organisms.

TABLE XII
UTILIZATION OF NORMAL BUTYRIC ACID AND FIXATION OF NITROGEN
BY CULTURE NO. 62

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed	Mgs. acid used per mg. nitrogen fixed
1	Control 0	Sterile	7.0-7.4	978	—	—	—
2	Control 0	Sterile	7.0-7.4	995	—	—	—
3	2	Pure	7.0-7.4	956	30	.09	334
4	2	Pure	7.0-7.4	878	108	1.25	86
5	5	Pure	7.0-7.4	706	280	2.73	102
6	5	Pure	7.0-7.4	669	317	4.15	76
7	7	Pure	7.0-7.4	439	547	2.59	212
8	12	Pure	9.0+	78	908	6.77	134
9	17	Contaminated	9.0+	61	925	4.95	186
10	17	Contaminated	9.0+	111	875	6.29	140

Utilization of iso-butyric acid.—Under the experimental conditions to which the cultures recorded in table XIII were subjected there was little or no indication that culture No. 62 could utilize iso-butyric acid in its metabolism. It is true that there was some decrease in the quantity of volatile acid present in the different cultures but the quantities were small. Besides, the non-inoculated sterile aerated controls, No. 2 and No. 3, showed practically the same decrease as inoculated flask No. 10, incubated the same length of time. In addition there was no perceptible change in the reaction, and the quantities of nitrogen fixed, if any, did not exceed the experimental error. Visible growth was also

questionable. It seems safe, therefore, to conclude that culture No. 62 could not utilize the calcium salt of iso-butyric acid under the conditions obtaining in these experiments.

TABLE XIII
UTILIZATION OF ISO-BUTYRIC ACID BY CULTURE NO. 62

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed
1	Control 0	Sterile	7.0-7.4	831	None	None
2	Control 17	Sterile	7.0-7.4	774	None	None
3	Control 17	Sterile	7.0-7.4	773	None	None
4	2	Pure	7.0-7.4	886	None	None
5	2	Pure	7.0-7.4	800	None	None
6	5	Contaminated	7.0-7.4	779	None	None
7	5	Pure	7.0-7.4	778	None	None
8	7	Contaminated	7.0-7.4	799	None	None
9	12	Pure	7.0-7.4	746	None	None
10	17	Contaminated	7.0-7.4	758	None	None

Utilization of normal valeric acid.—Not only was normal valeric acid not available to culture No. 62 but it actually was sufficiently toxic to kill all the introduced organisms within three days. This is the only instance in which any acid studied, other than formic, has actually killed the culture except when marked change in reaction occurred. There was of course no utilization of the acid or fixation of nitrogen. The slight loss of volatile acid previously referred to is evident in the data presented in table xiv.

*Utilization of monohydrated valeric acid.*¹—The data presented in table xv with regard to this acid are inconclusive. There is a distinct loss of acid, evidently not due to its removal through aeration, because uninoculated aeration controls No. 3 and No. 4 incubated one day longer than the longest incubated inoculated flask showed no loss in volatile acid. On the other hand, there was no perceptible change in reaction, and the quantities of nitrogen fixed, if any, were too small to detect, there being no

¹ Mono- and trihydrated valeric acids were iso compounds made by Merck and Co.

TABLE XIV

UTILIZATION OF NORMAL VALERIC ACID BY CULTURE NO. 62

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed
1	Control 0	Sterile	6.6-7.0	992	None	None
2	Control 0	Sterile	6.6-7.0	992	None	None
3	3	Sterile	6.6-7.0	988	None	None
4	6	Contaminated	6.6-7.0	890	None	None
5	11	Sterile	6.6-7.0	990	None	None
6	11	Sterile	6.6-7.0	913	None	None
7	18	Sterile	6.6-7.0	973	None	None
8	18	Sterile	6.6-7.0	979	None	None
9	32	Sterile	6.6-7.0	935	None	None
10	32	Sterile	6.6-7.0	951	None	None

TABLE XV

UTILIZATION OF MONOHYDRATED VALERIC ACID BY CULTURE NO. 62

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed
1	Control 0	Sterile	7.0-7.4	1055	—	None
2	Control 0	Sterile	7.0-7.4	1058	—	None
3	Control 33	Sterile	7.0-7.4	1064	—	None
4	Control 33	Sterile	7.0-7.4	1066	—	None
5	3	Pure	7.0-7.4	1051	10	None
6	6	Pure	7.0-7.4	944	117	None
7	11	Pure	7.0-7.4	911	150	None
8	11	Pure	7.0-7.4	972	89	None
9	18	Pure	7.0-7.4	899	162	None
10	18	Pure	7.0-7.4	907	154	None
11	32	Pure	7.0-7.4	871	190	None
12	32	Pure	7.0-7.4	933	128	None

difference in the nitrogen content of flasks No. 11 and No. 12 and sterile controls No. 3 and No. 4.

Utilization of trihydrated valeric acid.—Here again the quantities of volatile acid not recovered and increases in total nitrogen were not sufficient to be regarded as significant. If the quantities of acid disappearing are based upon aerated control No. 3 no losses are evident. Unfortunately, this sample became con-

taminated, and conclusions based upon it would not be entirely valid. On the other hand, if the quantities of acid recovered from the various flasks are compared with those recovered from controls No. 1 and No. 2, analyzed at the beginning of the experiment, small losses of acid are evident. What has been said with regard to losses of acid is equally true of increases in nitrogen. It is preferred, therefore, to regard it merely as a questionable possibility that culture No. 62 assimilates this acid qualitatively. The quantitative utilization of this acid, as well as of the monohydrated sample, is certainly small, if it occurs at all, compared to that of some of the other acid studied.

TABLE XVI
UTILIZATION OF TRIHYDRATED VALERIC ACID BY CULTURE NO. 62

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed
1	Control 0	Sterile	7.0-7.4	784	Utilization questionable	Fixation questionable
2	Control 0	Sterile	7.0-7.4	785		
3	19	Contaminated	7.0-7.4	726		
4	3	Pure	7.0-7.4	719		
5	3	Pure	7.0-7.4	776		
6	7	Pure	7.0-7.4	756		
7	7	Contaminated	7.0-7.4	752		
8	13	Pure	7.0-7.4	766		
9	13	Pure	7.0-7.4	732		
10	19	Contaminated	7.0-7.4	743		
11	19	Contaminated	7.0-7.4	756		

Utilization of normal caproic acid.—The data presented in table xvii show beyond question that the calcium salt of normal caproic acid may serve as a readily available source of organic material for culture No. 62. The samples analyzed after seven days' incubation, while still containing large quantities of volatile acid, showed marked changes in reaction, appreciable losses of acid, and fixation of nitrogen. The samples analyzed after thirteen days still contained appreciable quantities of acid but the hydroxyl-ion concentration had probably reached a point where growth was inhibited. This is further indicated by the failure to detect further losses of acid in the flasks incubated for a longer time, No. 10 and No. 11.

TABLE XVII

UTILIZATION OF NORMAL CAPROIC ACID BY CULTURE NO. 62

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed	Mgs. acid used per mg. nitrogen fixed
1	Control 0	Sterile	7.0-7.4	408	—	—	—
2	Control 19	Contaminated	9.0+	137	—	—	—
3	Control 19	Contaminated	7.0-7.4	359	—	—	—
4	3	Pure	6.6-7.0	351	57	1.02	56
5	3	Pure	7.0-7.4	374	34	.98	34
6	7	Pure	7.6-8.0	337	71	.50	142
7	7	Contaminated	7.8-8.2	260	148	2.16	69
8	13	Contaminated	9.0+	87	321	2.96	104
9	13	Contaminated	9.0+	75	333	Lost	—
10	19	Pure	9.0+	102	306	4.80	64
11	19	Pure	9.0+	109	299	—	—

Utilization of iso-caproic acid.—While the data presented in table XVIII may be regarded as inconclusive, they nevertheless indicate that iso-caproic acid is not available as an organic food for culture No. 62. There were slight losses of acid, but such losses were equally as marked from the non-inoculated, sterile, aerated controls as from any inoculated cultures. Further—

TABLE XVIII

UTILIZATION OF ISO-CAPROIC ACID BY CULTURE NO. 62

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed
1	Control 0	Sterile	7.0-7.4	560	Utilization questionable	Fixation questionable
2	Control 0	Sterile	7.0-7.4	533		
3	Control 19	Sterile	7.0-7.4	520		
4	Control 19	Sterile	7.0-7.4	497		
5	3	Pure	7.0-7.4	526		
6	3	Pure	7.0-7.4	—		
7	7	Pure	7.0-7.4	522		
8	7	Pure	7.0-7.4	505		
9	13	Pure	7.0-7.4	517		
10	13	Contaminated	7.0-7.4	493		
11	19	?	7.0-7.4	513		
12	19	Sterile	7.0-7.4	518		

more, there was no appreciable changes in reaction or detectable increases in the nitrogen content.

Summary of experiments with culture No. 62.—Under the experimental conditions to which culture No. 62 was subjected in the experiments herein reported the calcium salts of formic and normal valeric acids were strongly germicidal. Similar salts of acetic, propionic, normal butyric, and normal caproic acids served as readily available sources of organic food. The other salts tested, namely, iso-butyric, mono- and trihydrated valeric and iso-caproic, were either not available or utilized very slowly and in small quantities.

EXPERIMENTS WITH AZOTOBACTER VINELANDII

The culture of *Azotobacter vinelandii* used in these experiments (Culture No. 94) was obtained from N. R. Smith, of the Bureau of Plant Industry, U. S. Department of Agriculture. It grew vigorously upon soil-extract-mannitol agar, less vigorously upon beef-extract agar, and produced the typical green fluorescence upon the former medium. It also grew very abundantly in the liquid medium employed when dextrose, mannitol, and calcium salts of several of the fatty acids were supplied as the organic material.

Utilization of formic acid.—There was no evidence either in the qualitative or quantitative experiments of the ability of this organism to utilize calcium formate and therefore the quantitative data are omitted.

Utilization of acetic acid.—An examination of the data presented in table XIX should convince any one of the ability of this culture to utilize readily the calcium salt of acetic acid. A very marked change in the reaction, an almost complete disappearance of the acid accompanied by definite fixation of nitrogen, together with an abundance of visible growth, substantiate the above conclusions. In this experiment the hydroxylion concentration evidently reached a point that could not be tolerated by the organisms, most of them being dead when the flasks incubated for the longer period of time were examined.

Utilization of propionic acid.—*Azotobacter vinelandii* can readily assimilate propionic acid under the conditions obtaining in the

TABLE XIX

UTILIZATION OF ACETIC ACID AND FIXATION OF NITROGEN BY AZOTO-BACTER VINELANDII

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed	Mgs. acid used per mg. nitrogen fixed
1	Control	Sterile	7.0-7.4	876	—	—	—
2	Control	Sterile	7.0-7.4	859	—	—	—
3	4	Pure	7.0-7.4	783	84	-.06	—
4	4	Pure	7.0-7.4	790	77	.20	384
5	9	Pure	7.0-7.4	667	200	-.20	—
6	9	Pure	7.0-7.4	575	292	.12	440
7	14	Pure	7.0-7.4	423	444	.50	888
8	14	Pure	7.0-7.4	389	478	.96	498
9	24	Pure*	9.0+	3	864	1.60	540
10	24	Pure*	9.0+	3	864	3.06	282
11	33	Sterile	9.0+	5	862	2.36	366
12	33	Pure*	9.0+	11	856	2.22	386

* Very few living organisms.

experiment reported in table xx. Within three weeks the original 1.0 per cent of acid had practically disappeared. The abundance of visible growth, the marked increase in hydroxyl-ion concentration, and definite increases in the nitrogen content of the cultures are additional proof of the ability of the organism to assimilate this particular acid.

TABLE XX

UTILIZATION OF PROPIONIC ACID AND FIXATION OF NITROGEN BY AZOTO-BACTER VINELANDII

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed	Mgs. acid used per mg. nitrogen fixed
1	Control	Sterile	7.0-7.4	1009	—	—	—
2	Control	Sterile	7.0-7.4	999	—	—	—
3	7	Pure	7.0-7.4	790	214	.32	668
4	14	Pure	7.0-7.4	575	429	1.46	194
5	23	Pure	9.0+	16	988	5.92	168
6	23	Pure	9.0+	45	959	6.06	158
7	30	Pure	9.0+	7	997	4.46	224
8	30	Sterile	9.0+	10	994	4.02	247
9	39	Sterile	9.0+	7	997	3.12	319
10	39	Sterile	9.0+	7	997	2.36	422
11	46	Sterile	9.0+	4	1000	2.22	450
12	46	Sterile	9.0+	6	998	3.12	319

Utilization of normal butyric acid.—Some irregularities were exhibited in the growth in different culture flasks containing

normal butyric acid and inoculated with *Azotobacter vinelandii* despite all efforts to make the duplicate flasks as nearly identical as possible. These irregularities are reflected in the quantity of acid unrecoverable, the changes in reaction, and in the increases in nitrogen content as recorded in table XXI. However, these data unquestionably show a ready utilization of this acid by the culture in question. The rapidity of disappearance of the acid is not as great as with acetic and propionic acids, but the increases in nitrogen per unit of acid assimilated are greater.

TABLE XXI

UTILIZATION OF NORMAL BUTYRIC ACID AND FIXATION OF NITROGEN BY AZOTOBACTER VINELANDII

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed	Mgs. acid used per mg. nitrogen fixed
1	Control	Sterile	7.0-7.4	806	—	—	—
2	Control	Sterile	7.0-7.4	819	—	—	—
3	9	Pure	7.0-7.4	761	51	.12	424
4	9	Pure	7.0-7.4	750	62	.38	162
5	16	Pure	7.0-7.4	700	112	.76	148
6	16	Pure	7.0-7.4	700	112	.64	176
7	25	Pure	7.0-7.4	499	313	2.62	118
8	25	Pure	7.0-7.4	314	498	5.80	90
9	39	Sterile	9.0+	46	766	6.12	126
10	39	Pure	8.4-8.8	321	491	3.82	128
11	53	Pure	9.0+	51	761	6.12	124
12	53	Pure	8.8-9.2	411	401	3.56	112

Utilization of iso-butyric acid.—The data presented in table XXII indicate rather strongly that the calcium salt of iso-butyric acid is not nearly so readily available as is the corresponding salt of the normal acid. Even after forty-nine days over half of the original acid was still present, the reaction had changed only slightly, and the quantity of nitrogen fixed was small compared with that fixed where the normal acid was present. Besides, the visible growth (unmistakably present) was also small compared to that where acetic, propionic, or normal butyric acid was the source of organic food.

Utilization of normal valeric acid.—This acid apparently was not assimilated by *Azotobacter vinelandii* as readily as some of the lower members of the series. However, the figures presented in table XXIII show practically complete disappearance in flasks

TABLE XXII

UTILIZATION OF ISO-BUTYRIC ACID AND FIXATION OF NITROGEN BY
AZOTOBACTER VINELANDII

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed	Mgs. acid used per mg. nitrogen fixed
1	Control	Sterile	7.0-7.4	835	—	—	—
2	Control	Sterile	7.0-7.4	846	—	—	—
3	7	Pure	7.0-7.4	795	45	Lost	—
4	7	Pure	7.0-7.4	790	50	Lost	—
5	14	Pure	7.0-7.4	753	87	.26	334
6	14	Pure	7.0-7.4	Lost	—	.32	—
7	17	Pure	7.0-7.4	700	140	.12	—
8	17	Pure	7.0-7.4	728	112	.64	174
9	26	Pure	7.0-7.4	662	178	.64	278
10	26	Pure	7.0-7.4	634	206	.64	322
11	38	Pure	7.0-7.4	495	343	1.22	282
12	49	Pure	7.0-7.4	467	373	1.14	338

Nos. 9, 11, and 12. The more rapid assimilation in these instances, though, might have been partially due to the contaminating organisms. That there was, however, unmistakable utilization in the uncontaminated flasks, Nos. 7, 8, and 10, is proved by the decreased acid content, change in reaction, and increase in nitrogen content. Both the total quantity of nitrogen fixed and the relative quantity fixed per unit of acid assimilated were large, the latter being greater than for any lower member of the fatty acid series.

TABLE XXIII

UTILIZATION OF NORMAL VALERIC ACID AND FIXATION OF NITROGEN
BY AZOTOBACTER VINELANDII

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed	Mgs. acid used per mg. nitrogen fixed
1	Control	Sterile	7.0-7.4	892	—	—	—
2	Control	Sterile	7.0-7.4	883	—	—	—
3	7	Pure	7.0-7.4	810	77	.70	110
4	7	Pure	7.0-7.4	835	52	.70	74
5	14	Contaminated	7.0-7.4	770	117	.96	122
6	14	Contaminated	7.0-7.4	750	137	1.34	102
7	26	Pure	8.8-9.2	197	690	6.68	102
8	26	Pure	8.8-9.2	621	266	2.80	96
9	38	Contaminated	9.0+	54	833	6.76	122
10	38	Pure	7.4-7.8	414	473	6.56	72
11	49	Contaminated	9.0+	62	825	7.65	108
12	49	Contaminated	9.0+	60	827	11.98	70

Utilization of monohydrated valeric acid.—Growth in the presence of this acid was slow, and the total amount appeared to be much less than with the normal valeric or with the acids of smaller molecular weight. This was reflected in the rate and total disappearance of the acid as well as in the quantity of nitrogen fixed. It is also evident from the data recorded in table XXIV that slight, if any, change took place in the hydrogen-ion concentration. However, the amount of nitrogen fixed and acid consumed, coupled with the presence of visible growth, show conclusively that this acid can be assimilated by the culture in question, but probably not as readily as the other low molecular weight straight-chain, fatty acids, at least not under the conditions of these experiments.

TABLE XXIV

UTILIZATION OF MONOHYDRATED VALERIC ACID AND FIXATION OF NITROGEN BY AZOTOBACTER VINELANDII

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed	Mgs. acid used per mg. nitrogen fixed
1	Control	Sterile	7.0-7.4	989	—	—	—
2	Control	Sterile	7.0-7.4	1021	—	—	—
3	9	Pure	7.0-7.4	831	192	.64	300
4	9	Pure	7.0-7.4	830	175	.76	230
5	21	Pure	7.0-7.4	816	189	1.40	136
6	21	Pure	7.0-7.4	841	164	1.52	108
7	28	Pure	7.0-7.4	685	320	2.62	122
8	28	Pure	7.0-7.4	714	291	1.52	192
9	38	Pure	7.0-7.4	637	368	2.04	180
10	38	Pure	7.0-7.4	712	293	1.66	176
11	49	Pure	7.0-7.4	631	374	1.90	196
12	49	Pure	7.0-7.4	663	342	2.16	158

Utilization of trihydrated valeric acid.—What was said with regard to the monohydrated valeric acid also applies to the trihydrated, except that the quantities of acid assimilated and the quantities of nitrogen fixed were smaller. No perceptible change took place in the hydrogen-ion concentration, and only one-fifth of the acid was not recoverable after seven weeks of incubation. These facts would indicate that this acid is assimilable by *Azotobacter vinelandii* with somewhat more difficulty than either the normal or monohydrated valeric acids. The data are presented in table XXV.

TABLE XXV

UTILIZATION OF TRIHYDRATED VALERIC ACID AND FIXATION OF NITROGEN BY *AZOTOBACTER VINELANDII*

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed	Mgs. acid used per mg. nitrogen fixed
1	Control	Sterile	7.0-7.4	784	—	—	—
2	Control	Sterile	7.0-7.4	788	—	—	—
3	7	Pure	7.0-7.4	730	56	.38	148
4	7	Pure	7.0-7.4	753	33	.26	122
5	14	Pure	7.0-7.4	729	57	.26	218
6	14	Contaminated	7.0-7.4	714	72	.32	224
7	24	Pure	7.0-7.4	752	34	.50	68
8	24	Pure	7.0-7.4	708	78	.88	88
9	35	Pure	7.0-7.4	656	130	1.28	116
10	35	Pure	7.0-7.4	668	118	1.22	96
11	49	Contaminated	7.0-7.4	632	154	.76	202
12	49	Pure	7.0-7.4	628	158	.64	246

Utilization of normal caproic acid.—It would appear from the information recorded in table xxvi that normal caproic acid can be metabolized by *Azotobacter vinelandii* the most readily of any fatty acid tested. Within four days a very heavy growth was evident, half the acid added to the medium had disappeared, the reaction had become alkaline to thymol-blue, and marked fixation of nitrogen had taken place. Within nine days the volatile acid had reached the minimum recorded for any incubation period. Furthermore, the quantity of nitrogen fixed per

TABLE XXVI

UTILIZATION OF NORMAL CAPROIC ACID AND FIXATION OF NITROGEN BY *AZOTOBACTER VINELANDII*

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed	Mgs. acid used per mg. nitrogen fixed
1	Control	Sterile	7.0-7.4	408	—	—	—
2	Control	Sterile	7.0-7.4	381	—	—	—
3	4	Pure	9.0+	267	128	3.92	32
4	4	Pure	9.0+	180	215	4.66	46
5	9	?	9.0+	39	356	5.86	60
6	9	Pure	9.0+	41	354	5.48	64
7	16	Pure	9.0+	32	363	6.06	60
8	16	Pure	9.0+	28	367	6.62	56
9	28	Contaminated	9.0+	27	368	5.28	70
10	28	Contaminated	9.0+	31	364	6.50	56
11	37	Contaminated	9.0+	35	360	8.34	82
12	37	Pure	9.0+	35	360	5.10	70

unit of acid assimilated was greater than for any other acid tested quantitatively. This would seem to indicate that increasing the size of the molecule does not necessarily decrease its availability.

Utilization of iso-caproic acid.—This acid was, according to the data presented in table XXVII, readily assimilated, though the rate of growth, acid utilization, and nitrogen fixation were not equal to the corresponding rates where normal caproic acid was the sole organic food supplied. Similarly, the ratio between acid consumed and nitrogen fixed was twice as wide as for the normal acid. The iso compound then, it would seem, is not only less readily metabolized but can also not be used as economically as the straight-chain molecule of this acid.

TABLE XXVII

UTILIZATION OF ISO-CAPROIC ACID AND FIXATION OF NITROGEN BY
AZOTOBACTER VINELANDII

Flask No.	Days incubated	Purity	Approximate reaction expressed as P_H	Mgs. acid recovered	Mgs. acid utilized	Mgs. nitrogen fixed	Mgs. acid used per mg. nitrogen fixed
1	Control	Sterile	7.0-7.4	417	—	—	—
2	Control	Sterile	7.0-7.4	409	—	—	—
3	7	Pure	7.8-8.2	323	90	.50	180
4	7	Pure	7.8-8.2	272	131	.90	144
5	16	Pure	8.2-8.6	90	323	2.28	142
6	16	Pure	8.2-8.6	75	358	2.16	156
7	28	Pure	9.0+	23	390	2.54	154
8	28	Pure	9.0+	22	391	3.18	122
9	37	Pure	9.0+	77	336	3.70	90
10	37	Pure	9.0+	23	390	2.80	140
11	51	Sterile	9.0+	17	396	2.54	156
12	51	Pure*	9.0+	15	398	2.68	148

* Only four colonies developed on two plates.

Summary of experiments with Azotobacter vinelandii (culture No. 94).—*Azotobacter vinelandii* is capable of growing in a medium containing as the only organic material the calcium salt of the following fatty acids: acetic, propionic, normal butyric, isobutyric, normal valeric, iso-valeric (monohydrated valeric and trihydrated valeric), normal caproic, and iso-caproic. This organism failed to grow under similar conditions in the presence of calcium formate.

The rate of growth varies with the different calcium salt added

to the medium, being very rapid with normal caproic and very slow with all of the iso acids tested. When growth takes place there is an increase in the nitrogen content of the medium and a decrease in the quantity of volatile acid.

The quantity of nitrogen fixed in the presence of any given acid corresponds more or less closely with the quantity of acid disappearing. The increase in nitrogen per unit of acid consumed by the organisms varies with different acids. If only normal acids are considered the quantity of nitrogen fixed per unit of acid decomposed increases as the molecular weight of the acid increases. Growth, disappearance of acid, and increase in nitrogen are not as rapid where iso acids are added to the medium as when the acid is a normal compound.

EXPERIMENTS WITH OTHER CULTURES

Since there were a number of instances in which the results secured with cultures No. 62 and No. 94 did not agree, it seemed desirable to extend somewhat similar tests to other cultures. In order to secure very active nitrogen-fixing strains for these tests the experiment recorded in table XXVIII was arranged. It was also hoped that this experiment would show to what extent vigorous growth in liquid media, utilization of dextrose, and fixation of nitrogen could be correlated.

Erlenmeyer flasks of 300 cc. capacity containing 1.0 per cent dextrose medium were prepared and inoculated heavily with the cultures indicated. One of the triplicate flasks was used for qualitative sugar tests. Growth observations were recorded from the remaining two flasks and after fifteen days' incubation the quantity of nitrogen fixed was determined.

Since the dextrose had completely disappeared in all but two instances and the original quantities in the various cultures were identical, the total nitrogen figures represent approximately the quantity of nitrogen fixed per 1000 mgs. dextrose consumed. It will be noted that with few exceptions the quantities of nitrogen fixed do not vary very widely. The smallest quantity fixed in any instance where complete disappearance of sugar had taken place was 2.64 mgs. while the largest was 11.46 mgs. Most of the cultures showed a fixation of about 8 to 10 mgs. per 1000

TABLE XXVIII

THE ABILITY OF VARIOUS AZOTOBACTER CULTURES TO GROW IN THE PRESENCE OF, AND TO ASSIMILATE, DEXTROSE, AND THEIR RELATIVE ABILITY TO FIX NITROGEN

Culture No.	Quantity of growth after varying periods of incubation			Presence of dextrose after varying periods of incubation		Nitrogen fixed per 100 cc. medium
	2 days	4 days	8 days	8 days	14 days	
219	?	?	+	Abundant	0	8.14
187(a)	+	++++	++++	0	0	10.14
55	0	0	++	Abundant	0	9.16
209	++	++++	++++	0	0	9.92
C	++	++++	++++	0	0	10.62
94	+++	++++	++++	0	0	8.00
204	?	+++	++++	0	0	9.16
220	?	+	++	Abundant	0	9.10
15	?	?	?	Abundant	Abundant	2.64
19	+++	++++	++++	0	0	10.00
209	+	+++	++++	0	0	9.54
27	?	?	+	0	0	10.06
215(4)	++	+++	+++	0	0	8.66
II	++	+++	+++	Abundant	0	10.06
216	+++	+++	++++	0	0	9.04
144	?	?	++	0	0	10.38
16	?	?	++	Abundant	Abundant	2.74
103(4)	?	++++	++++	0	0	8.92
226(B)	+	+	++++	0	0	7.04
188	++++	++++	++++	0	0	8.28
86	+++	++++	++++	0	0	11.46
97	+++	+++	++++	0	0	9.30
95	++	+++	+++	0	0	7.06
B	?	?	+	Abundant	0	10.70
7	?	+	++	0	0	7.96
48	?	+	+++	Abundant	0	10.32
11(2)	++	+++	+++	0	0	8.92
56	?	+	++	0	0	9.16
S-2	0	?	+	Abundant	0	6.88
194	0	+	++	0	0	9.80
185	+	+	+++	0	0	8.08
165	?	+	++++	0	0	10.06
25	?	+++	+++	0	0	8.94
44	0	0	+	Abundant	0	8.46
I	0	+	+++	0	0	8.66
218	?	+++	+++	0	0	8.84
III	+	+++	+++	0	0	6.56
215(2)	+	+++	+++	0	0	6.24
178	0	?	++	0	0	10.62
198(1)	+	+++	+++	0	0	8.78
15(1)	0	0	+	0	0	7.64
Control	0	0	0	Abundant	Abundant	—
Control	0	0	0	Abundant	Abundant	—
Control	0	0	0	Abundant	Abundant	—

mgs. of dextrose or 100 to 120 parts of sugar consumed for each part of nitrogen fixed. This would indicate that the organic

food available, provided the particular culture in question is capable of utilizing it, is possibly of more significance in determining the quantity of nitrogen fixed than is the culture. There was, however, a rather marked variation in the rate at which various cultures consumed the dextrose.

Another very evident fact is that the quantity of visible growth cannot be taken as necessarily indicating the relative fixation of nitrogen. Cultures Nos. 219, 55, 220, 27, 144, B, 44, and 178 all showed a relatively small visible growth, yet they were among the most active nitrogen-fixing strains. Cultures Nos. 15 and 16 were evidently unable to utilize dextrose very readily. It is possible, though, that they may have fixed as much nitrogen per unit of dextrose consumed as any other culture.

From the cultures tested in the experiment just described nine, Nos. 7, C, 165, II, 188, 194, 187, 178, 218, and in addition R, were chosen for a comparative study of their ability to utilize the various acids studied in previous experiments. These particular cultures were selected both because of their active nitrogen-fixing ability and because they were secured from such widely varying conditions. Furthermore, they exhibited rather marked variations in cultural characteristics, indicating that they represented different strains or possibly species. In this experiment 100 cc. of the medium were placed in 750-cc. or 1000-cc. flasks, thus giving excellent aeration.

The rate of visible growth is indicated in table XXIX, while the quantities of acid consumed and nitrogen fixed are recorded in table XXX.

The data presented in tables XXIX and XXX tend to confirm the previous results secured from cultures No. 62 and 94 in that rather marked variations are exhibited in the ability of various cultures to grow in the presence of calcium salts of different organic acids as the only organic matter present.

All ten of the cultures readily assimilated dextrose, though culture No. II probably with somewhat more difficulty than the others. All grew in the presence of the calcium salts of acetic, propionic, normal valeric, normal butyric and normal caproic acids, the latter apparently being more easily metabolized than

TABLE XXIX (continued)

Culture No.	Growth in the presence of							
	Acetic acid	Propionic acid	Normal butyric acid	Iso-butyric acid	Normal valeric acid	Mono-hydrated valeric acid	Tri-hydrated valeric acid	Normal caproic acid
11	?	0	0	0	?	?	0	?
	+	0	0	0	?	?	0	+
	+	?	0	0	+	?	0	+
	+	+	?	?	+	?	0	+
188	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+
194	+	+	?	0	+	0	0	+
	+	+	?	?	+	+	0	+
	+	+	+	?	+	+	?	+
	+	+	+	+	+	+	?	+
187	+	+	?	+	+	?	?	+
	+	+	+	+	+	?	?	+
	+	+	+	+	+	?	?	+
	+	+	+	+	+	?	?	+
178	+	+	+	+	+	+	?	+
	+	+	+	+	+	+	?	+
	+	+	+	+	+	+	?	+
	+	+	+	+	+	+	?	+

• (a) Observations made after 5 days incubation at 30° C.
 (b) Observations made after 8 days incubation at 30° C.
 (c) Observations made after 13 days incubation at 30° C.
 (d) Observations made after 23 days incubation at 30° C.
 (e) Observations made after 25 days incubation at 30° C.

TABLE XXX
GROWTH, UTILIZATION OF VARIOUS FATTY ACIDS, AND NITROGEN FIXED BY TEN DIFFERENT CULTURES OF AZOTO-
BACTER

Culture No.	Acetic acid	Propionic acid	Normal butyric acid	Iso-butyric acid	Normal valeric acid	Mono-hydrated valeric acid	Tri-hydrated valeric acid	Normal caproic acid	Iso-caproic acid	Dextrose
Visible growth (35 days incubation)										
R	++	++	+	?	++	++	+	++	0	++
7	++	++	++	?	++	?	?	++	0	++
C	++	++	++	?	++	?	?	++	0	++
218	++	++	++	++	++	++	+	++	0	++
165	++	++	++	++	++	++	+	++	0	++
11	++	++	++	++	++	++	0	++	0	++
188	++	++	++	++	++	++	+	++	0	++
194	++	++	++	++	++	++	+	++	0	++
187	++	++	++	++	++	++	?	++	0	++
178	++	++	++	++	++	++	?	++	0	++
Mgs. acid consumed										
R	883	618	276	71	378	152	-33	393	7	818
7	884	646	300	101	374	165	16	362	15	818
C	892	372	375	131	361	84	16	287	1	818
218	896	652	268	142	382	176	143	360	28	818
165	434	644	367	72	374	149	24	379	2	818
11	388	284	251	99	246	116	47	163	15	528
188	582	621	686	247	371	83	35	271	41	818
194	685	547	289	29	370	77	-51	247	1	818
187	385	386	189	247	375	143	7	393	21	818
178	379	391	308	70	365	57	-24	379	16	818

TABLE XXX (continued)

Culture No.	Acetic acid	Propionic acid	Normal butyric acid	Iso-butyric acid	Normal valeric acid	Mono-hydrated valeric acid	Tri-hydrated valeric acid	Normal caproic acid	Iso-caproic acid	Dextrose
Mgs. nitrogen fixed										
R	3.19	2.29	2.29	.64	2.68	1.14	.26	3.06	.06	3.32
7	1.53	2.55	2.29	.77	3.82	1.02	.00	3.32	.00	4.72
C	2.68	1.53	2.04	.89	3.44	1.02	.77	4.08	.00	6.42
218	1.52	1.27	1.27	3.52	1.65	.77	1.14	3.82	.00	3.06
165	1.14	2.04	2.04	.51	2.55	.89	.12	3.44	.00	6.50
II	.77	1.02	1.02	.51	2.43	.64	.12	1.53	.00	5.10
188	1.53	3.32	3.82	.77	2.55	1.02	.00	4.08	.00	8.16
194	1.53	2.80	2.04	.64	1.53	1.27	.00	3.32	.00	6.42
187	1.40	1.40	1.32	.51	2.04	1.53	.00	3.82	.00	5.61
178	1.53	1.78	1.65	.77	1.78	.89	.00	3.95	.00	8.67
Mgs. acid used per mg. nitrogen fixed										
R	277	270	121	109	141	133	—	128	—	247
7	578	253	131	131	98	162	—	109	—	173
C	833	243	135	147	115	82	—	70	—	127
218	685	613	211	—	231	229	—	95	—	267
165	381	315	180	141	147	167	—	111	—	126
II	463	278	246	194	101	181	—	107	—	103
188	367	187	179	321	143	81	—	66	—	100
194	448	231	141	—	241	61	—	74	—	127
187	275	276	143	484	184	93	—	103	—	164
178	248	220	187	91	205	77	—	96	—	94
Average	396	270	167	202	160	117	—	96	—	153
Mgs. nitrogen fixed per gm.	2.53	3.58	6.00	4.95	6.25	8.5		10.42		6.54
Mgs. nitrogen fixed per calory	.72	.72	1.01	.83	.93	1.27		1.45		1.73

any of the others, with valeric ranking next. It is quite evident, then, that increasing the size of the molecule does not decrease the availability. Not one of the cultures seemed capable of growing when the calcium salt of iso-caproic acid was the sole organic compound present. Growth with the calcium salts of iso-butyric or with the two hydrated valeric acids (the only organic materials present) was usually either very slow or absent. Culture No. 188 seemed to utilize a wider variety of acids with more ease than any other, its growth being recorded as + after three weeks with all the different acids except iso-caproic.

As previously pointed out, the quantity of visible growth is not necessarily associated with the quantity of nitrogen fixed or the quantity of organic material consumed. This is again evident if the visible growth as recorded in table XXIX is compared with the quantity of acid utilized and nitrogen fixed as recorded in table XXX.

The quantitative data presented in table XXX show very definitely that all ten of the strains of *Azotobacter* used in this experiment can utilize, as a source of organic food for nitrogen-fixing purposes, all the acids tested with the exception of trihydrated valeric and iso-caproic. Cultures C and 218 apparently utilized the trihydrated valeric acid. The iso compounds, though, cannot be assimilated as readily as the straight-chain molecules. Since this experiment could not be repeated no effort will be made to analyze critically the results secured with the individual cultures. It is quite evident that some of the cultures can assimilate certain acids very much more readily than can other cultures. Furthermore, some were capable of fixing very much more nitrogen per unit of acid with a given acid than were others.

The milligrams of acid consumed per milligram nitrogen fixed, the nitrogen fixed per gram acid, and the quantity of nitrogen fixed per calory of contained energy for the various acids were averaged and recorded at the end of table XXX. In a general way this summary agrees with the results secured with *Azotobacter vinelandii*. The quantity of nitrogen fixed per gram of acid metabolized increases as the molecular weight increases. In fact the increase in quantity of nitrogen fixed is more rapid

than the increase in heat of combustion, indicating a more efficient use of the energy contained in the larger molecules.

The conclusion seems justified, then, that the calcium salts of acetic, propionic, normal butyric, normal valeric, and normal caproic acids can be very readily assimilated by numerous strains of *Azotobacter*, while the ability to assimilate iso-caproic acid is rather limited among these organisms. The other acids tested, namely, iso-butyric and iso-valeric acids, while capable of being assimilated by most of the cultures tested, are certainly not as readily available as are acetic, propionic, normal valeric, and normal caproic.

As a further check on the relative availability of the various acids the protocol arranged in table xxxi was carried out experimentally. The acids used in this experiment were all from new lots and, because of limited quantities available, only approximately 0.5 per cent concentrations were used. Even with 0.5 per cent, large quantities of heptylic and caprylic acids remained undissolved in the culture medium. Erlenmeyer flasks of 300 cc. capacity containing 50 cc. of the medium and inoculated in duplicate served as cultures.

In addition to the fatty acids a number of polyhydric alcohols of varying molecular weights and some of identical molecular weights but varying configuration were used in order to see if similar variability in assimilability, as observed for acids, existed for alcohols.

Culture No. 94 again demonstrated its superiority over either No. 178 or No. 218 to utilize a variety of fatty acids, and also to metabolize more readily the alcohols. Both the latter cultures again failed to utilize any of the iso compounds readily and some not at all. These data, secured from entirely different batches of acids, tend to substantiate the observations noted from experiments recorded earlier in this paper. One new acid, di-methyl-ethyl-acetic, was added but apparently was not even assimilated by culture No. 94.

The same variability as regards the utilization of different acids by different strains of *Azotobacter* is also evident when the alcohols are considered. Of the eight polyhydric alcohols tested, only sorbitol and mannitol were readily metabolized by

all three cultures, the former more rapidly than the latter. Dulcitol, an isomer of sorbitol and mannitol, apparently cannot be utilized by any of the three cultures. Inositol, a six carbon hexahydric ring alcohol, was readily utilized by culture No. 94, with more difficulty by culture No. 218, and not at all by culture No. 178. The same was true of glycerol. The four carbon alcohol of this series, erythritol, was utilized only by culture No. 94. The two and five carbon numbers, ethylene glycol and adonitol, were apparently not available to any of the three cultures. This fact is rather interesting since Mockridge obtained the maximum fixation in her experiments where ethylene glycol was the sole source of energy. Apparently the configuration of the molecule plays an important role in determining the ability of a given strain of *Azotobacter* to utilize a compound.

DISCUSSION

Attention has already been called to the salient facts brought out in the various experiments in the summaries accompanying the individual tables. In the limited discussion to follow an effort will be made merely to correlate these various points with the special object of trying to see if satisfactory answers to the original questions propounded in the introduction can be made.

In the first place, it is frankly admitted that certain procedures followed in some of the earlier experiments did not prove as satisfactory as had been hoped. This was true of the aeration in that the quantitative acid and nitrogen data did not, for some unknown reason, check as well as did later experiments. Because of these irregularities as much significance is not attached to the data secured in connection with those experiments as to those in which culture No. 94 was employed.

Secondly, such variation in the ability of different cultures to utilize different organic food substances was not anticipated or the experiments would have been confined entirely to identified cultures, thereby making it possible to compare results here reported with results obtained by other investigators. This desirability was realized in time to make use of known cultures in the major portion of these experiments, and data obtained with known organisms are regarded as much more significant.

In the third place the inadequacy of the data, in many or possibly all instances, from a quantitative point of view, is realized. It is believed, however, that in this respect they are equal or superior to any thus far reported. Where quantitative determinations, such as were employed in these experiments, are necessary the accumulation of mass data is a slow process. The limited data available are presented not as definite proof but rather as indicating certain tendencies, and it is hoped that others may see fit to conduct experiments along similar lines, thus bringing about the accumulation of sufficient data to justify definite conclusions.

It is desired to call attention again to, and to emphasize, the danger of applying the findings from a study of one strain of *Azotobacter* to other strains. If there is any one thing indicated by the experiments reported in this paper it is the marked variability in the metabolism of organic compounds of different strains or species in this group of organisms. This fact makes it rather difficult to compare results that have been reported from one laboratory with those from another, because frequently no indication whatsoever has been given as to the origin or identification of the culture studied. In the future much greater emphasis should be placed upon the identity of the culture being studied. For the reason just set forth it is believed that very little would be gained by a comprehensive review of investigations dealing with the utilization of various organic substances by this group of organisms.

A cursory perusal of the literature dealing with *Azotobacter* will impress one with the great variety of organic compounds that may be assimilated by these organisms. Also it brings out the wide variation in efficiency with which such compounds can be used when the quantity of nitrogen fixed in their presence is the criterion by which such efficiency is judged.

To illustrate the points suggested in the preceding paragraph the reader is referred to table xxxii. This table is an adaptation of one recently used by Bonazzi ('26) enlarged to include the work of Mockeridge and a few examples from the data previously presented in this paper.

Over fifty separate and distinct organic compounds are here

TABLE XXXII
RELATIVE FIXATION OF NITROGEN PER GRAM ORGANIC FOOD OBSERVED BY DIFFERENT INVESTIGATORS

Organic compound in medium	Mgs. nitrogen fixed per gram organic material						
	Lohnis and Pillai—impure		Hoffmann and Hammer		Krainsky	Mockeridge	Gainey
	+CaCO ₃	-CaCO ₃	Impure	Pure			
Polysaccharides:							
Starch	3.36	3.50			Pure	Pure	Pure*
Dextrine	7.18	7.58	1.72	13.40	0.40	5.93	
Inulin	7.72	7.58	1.35	10.85	1.20	6.62	
					5.80	9.76	
Gums:							
Gum arabic						6.13	
Gum tragacanth						9.13	
Sugars:							
Raffinose			2.23	5.35	1.60	7.28	
Saccharose	8.60	5.90	0.93	11.70	0.00	7.55	
Maltose	7.44	7.86	0.74		2.80	3.39	
Lactose	9.12	8.88	4.64	7.20	0.80	10.32	
Levulose	8.52	8.80	1.68	10.30	5.55	6.20	
Galactose	7.86	7.44	1.16	7.35	0.67	6.57	
Dextrose	4.62	4.36	1.65	8.95	1.35	9.00	8.85
Xylose	9.54	9.40		4.55			
Mannose				7.90			
Arabinose	7.62	7.34		10.00	0.60	9.28	
Alcohols:							
Mannitol	9.40	9.96	4.33	14.40	5.70	11.62	
Erythritol					0.00	4.88	
Glycerol	4.78	1.68		5.05	2.40	5.00	
Ethylene glycol						16.74	
Methyl					0.00	2.10	
Ethyl					1.00	4.02	
Propyl						9.02	
Iso-butyl						4.69	

TABLE XXXII (continued)

Organic compound in medium	Mgs. nitrogen fixed per gram organic material					
	Löhnis and Pillai—Impure		Hoffmann and Hammer		Krainsky	Mockeridge
	+CaCO ₃	-CaCO ₃	Impure	Pure		
Salts of fatty acids:						
Ca-formate						Pure
Ca-acetate						1.47
Na-propionate	1.10	0.96			3.20	3.77
Ca-propionate						
Ca-butyrate	0.02	0.16			0.00	5.16
Ca-butyrate (iso)						6.08
Ca-valerate (normal)						
Ca-valerate (monohydrated)						5.66
Ca-valerate (trihydrated)						6.52
Ca-caproate						3.53
Ca-caproate (iso)						10.71
						5.99
						7.49
						17.87
						7.57
Salts of other organic acids:						
Na-succinate	2.96	2.82				
Ca-succinate						
Na-citrate	1.42	1.00			1.00	8.60
Ca-lactate	2.49	2.22				6.44
K-oxalate	0.12	0.26				12.01
Na-tartrate	5.06	2.82				
Ca-tartrate						4.54
Ca-racemate						2.77
Ca-malonate						5.32
Ca-mucate						6.79
Ca-fumarate						2.00
Ca-maleate						1.88
Ca-glycollate						1.75
Ca-malate						5.19

* Data for dextrose secured with culture No. 62 (*Azotobacter chroococcum*). Data for Ca-formate from large number of cultures including *Azotobacter chroococcum* and *Azotobacter vinelandii*. Other data are for culture No. 94 (*Azotobacter vinelandii*).

recorded as being assimilable by *Azotobacter*. Among these are representatives of classes of organic compounds possessing, in many instances, very few characteristics in common. Furthermore, the compounds here listed are only those reported by five of the many investigators in this field and are by no means intended to represent all compounds that have been tested and found capable of supplying the organic needs of members of the *Azotobacter* group of organisms. Among the carbohydrates are examples of polysaccharides, gums, and sugars. Among the latter are tri-, di-, and mono-saccharoses as well as hexose and pentose sugars. Alcohols are represented by mono-, di-, tri-, tetra-, and hexa-hydrox compounds, also by straight-chain and iso arrangements of the carbons, with the additional variations in polarity of inactive, dextro-, and levo-rotary molecules. There are also twenty-five salts of organic acids, including representatives of a number of dissimilar groups. There would seem to be no question, then, but that among the species of *Azotobacter* there are members capable of utilizing a very wide variety of organic compounds as the only organic requirement for the fixation of nitrogen. Not only is this true but the same strain of organisms can function as a nitrogen fixer when supplied with a wide variety of compounds.

When it comes to the comparative efficiency with which these various compounds can be used, measured by quantitative gains in nitrogen, the data are too inadequate to permit of drawing any very definite conclusions. The work with impure cultures would have to be eliminated from consideration. This leaves only a few compounds that have been tested by two or more investigators. Of these ten may be selected that were studied in common by Hoffmann and Hammer ('10), Krainsky ('08), and Mockeridge ('15). The quantity of nitrogen fixed per gram of material as reported by these investigators is indicated in fig. 3.

The data plotted may not be very accurate, since there is no indication of quantitative determinations of the residual organic material having been made, except that Mockeridge states that qualitative tests showed complete absence of the organic compound. Furthermore, one is forced to assume that the original

1.0 per cent of material added remained quantitatively unaltered during the process of sterilization, a condition that certainly might not obtain in all cases. In this connection it is believed that the policy followed in experiments herein reported, of making quantitative determinations on controls submitted to the same treatment as the cultures, is a much safer procedure for ascertaining the quantity of the organic compound available to the organisms.

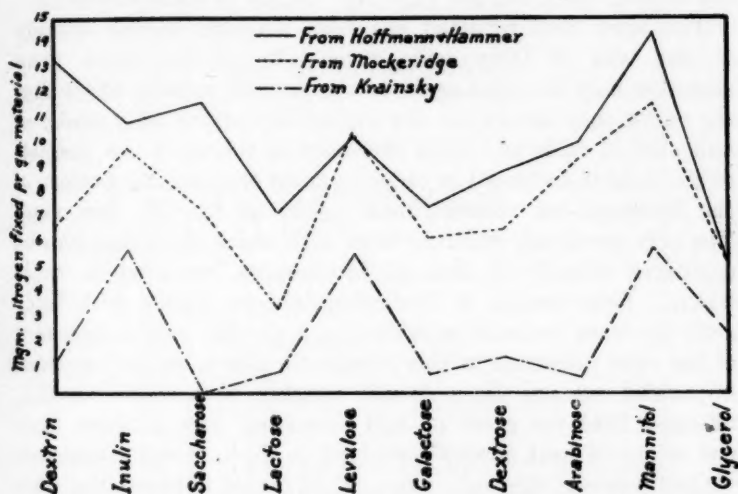


Fig. 3. Showing fixation of nitrogen per gram of organic material, secured by different investigators.

In spite of the numerous possibilities for errors the curves in fig. 3 show a marked qualitative similarity. The absolute quantities of nitrogen fixed per gram of material vary very widely. This might be taken to indicate a variation in the efficiency of the cultures, but, as mentioned in the preceding paragraph, it may merely mean that the organic material was not used up quantitatively in, for example, Krainsky's experiments.

If it is assumed that in all instances approximately the same quantity of organic material was available, and that it was used up quantitatively, then we would be justified in concluding that there are wide variations in the efficiency with which different

cultures utilize the same compounds and also a marked contrast in the efficiency with which the same culture used different organic materials in the fixation of nitrogen. There are strong indications, however, that different cultures may utilize many of the same compounds with approximately the same relative efficiency. Attention was called to this in connection with the experiment reported in table XXIII, in which out of forty cultures tested the quantity of nitrogen fixed, when dextrose served as the organic food, did not vary very widely in most instances.

The work here reported has been confined almost entirely to the salts of fatty acids, principally calcium salts. And attention may be called again to the possible marked effect that the cation may have upon the availability of the fatty acids, as indicated in table IX. It is necessary of course to use the salt of any acid to be tested in order to avoid the inhibiting effect of the hydrogen-ion concentration produced by the free acid. The only previously reported work with which these data can be compared directly is that of Mockeridge, recorded in table XXXII. That portion of Mockeridge's data dealing with fatty acids has been recorded in table XXXIII parallel with a summary of the data presented in this paper. In this table are recorded in parallel columns the molecular weights, heat of combustion, nitrogen fixed per gram of acid consumed, and nitrogen fixed per calory of heat energy contained in the material consumed.

Qualitatively, the only point of difference between the data presented here and those reported by Mockeridge is in the utilization of formic acid. In no instance have either quantitative or qualitative evidence of the growth of any culture of *Azotobacter* in the presence of a salt of formic acid been observed in this work.

Quantitatively, the data agree in showing that as the molecular weight or heat of combustion increases, the quantity of nitrogen fixed per unit of acid consumed increases. This is brought out clearly in fig. 4. There is, however, a difference, possibly significant, in the type of curves plotted from the two sets of data as shown in fig. 4 where the nitrogen fixed is plotted against molecular weight, or heat of combustion, since both increase arithmetically in the compounds as arranged in the figure.

TABLE XXXIII
MOLECULAR WEIGHT, HEAT OF COMBUSTION AND RELATIVE NITROGEN FIXED PER GRAM AND PER CALORY OF ORGANIC ACIDS BY CULTURE NO. 94 (AZOTOBACTER VINELANDII)

Acid	Molecular weight	Heat of combustion	Heat of combustion calories per gram	Mgs. nitrogen fixed per gram	Mgs. nitrogen fixed per calory	Mgs. nitrogen fixed per gm. (Mockeridge)	Mgs. nitrogen fixed per calory (Mockeridge)
Formic	46	61.7	1.34	0.00	0.00	1.47	1.10††
Acetic	60	209.4	3.49	2.60*	0.74	3.77	1.09
Propionic	74	367.4	4.97	5.66†	1.14	5.16	1.04
Butyric	88	524.4	5.96	8.52‡	1.43	6.08	1.02
Valeric	102	681.6	6.68	10.71§	1.58		
Caproic	116	830.2	7.16	17.87§	2.49		
Iso-butyric	88	524.4††	5.96	3.53*	0.60		
Mono-hydrated-valeric	102	681.6††	6.68	5.99§	0.90		
Tri-hydrated-valeric	102	681.6††	6.68	7.49**	1.12		
Iso-caproic	116	830.2††	7.16	7.57*	1.06		
Dextrose	180	677.2	3.76	8.85	2.33	6.57	1.75

* Average of 5 determinations.

† Average of 3 determinations.

‡ Average of 8 determinations.

§ Average of 10 determinations.

** Average of 7 determinations.

†† Assumed to be same as for normal compounds.

‡‡ Recalculated for heats of combustion recorded in table. Mockeridge used different heat of combustion values.

This difference is shown more strikingly in fig. 5 in which the nitrogen fixed is plotted against a unit of energy contained in the compound.

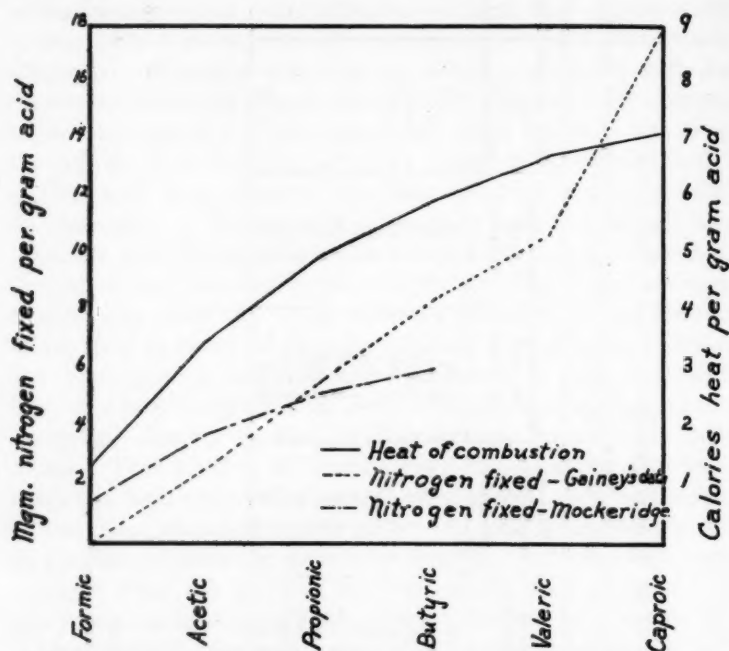


Fig. 4. Comparison between nitrogen fixation and heat of combustion per gram acid.

If the quantity of nitrogen fixed were proportional to the heat of combustion, then in fig. 4 all three curves should run parallel, while in fig. 5, the two lines should coincide and lie horizontal to the base line.

In fig. 4 the nitrogen-fixation curve plotted from Mockeridge's data tends to diverge from the curve for heat of combustion, and in fig. 5 it approaches the abscissa. Such curves would indicate that as the molecular weight increases the quantity of nitrogen fixed per unit of energy decreases. The decrease indicated in Mockeridge's data is very slight and is probably within the limit of error. On the other hand, the nitrogen fixation curve

in fig. 4 based upon new data starts well below the heat of combustion curve and actually crosses it with a marked upward tendency rather than a tendency to flatten out, while in fig. 5, starting on the base line, the curve continually, though not uniformly, rises from formic to caproic acid. Such curves indicate that as the molecular weight increases the organism is capable of utilizing the contained energy more efficiently. Atten-

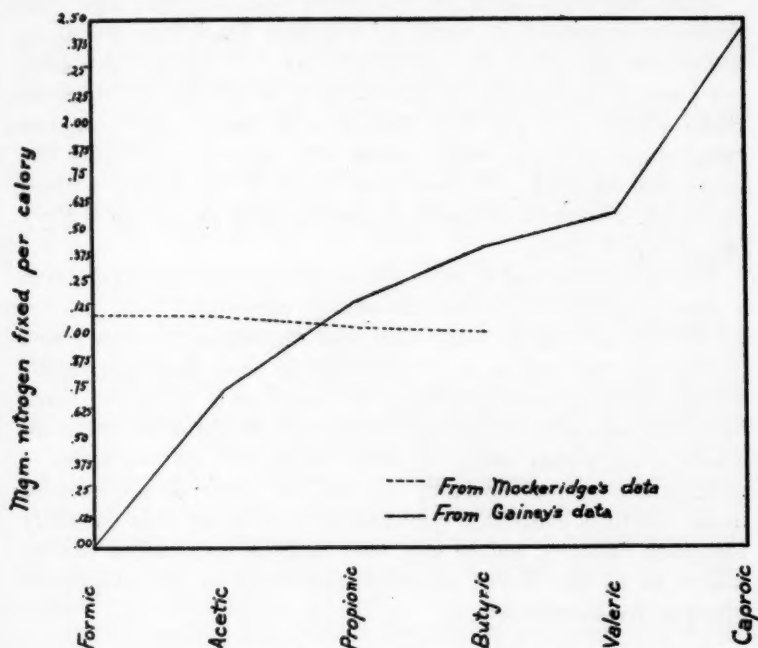


Fig. 5. Showing fixation of nitrogen per calory heat of combustion.

tion has previously been called to the fact that growth has been uniformly more rapid in caproic, and frequently in valeric acid, than in the acids of lower molecular weights.

The data serving as a basis for figs. 4 and 5 were secured from normal acids. In no instance has the iso acid appeared to be used as rapidly as the corresponding straight-chain compound. No explanation is offered as to the reason for this unless it is

that the straight-chain compounds are more frequently encountered by the organisms in nature and as a result they have become better adapted for metabolizing substances of this type. The data presented in table XXXIII indicate the same tendency on the part of *Azotobacter vinelandii* to utilize more efficiently, though not to the same degree, the iso acids of a higher molecular weight.

When the fatty acids are compared with dextrose and their efficiency measured in terms of nitrogen fixed, two points in connection therewith are worthy of note. If the nitrogen fixed per gram of material be used as a basis for comparison dextrose ranks about on a par with butyric acid, acetic and propionic being much inferior, while valeric and caproic, especially the latter, are superior. On the other hand, if the energy content be used as a basis for comparison, then dextrose ranks only slightly below caproic acid.

The data presented in table XXXIII are inconclusive in showing a close correlation between the energy content or heat of combustion of an organic compound and the efficiency with which *Azotobacter* can utilize it as the organic food required; nevertheless, there is more indication of a correlation when compared upon this basis than simply upon the actual weight of the material. There is an urgent need for much more data on the series of compounds herein reported, as well as other series, secured under carefully controlled quantitative conditions with definitely identified cultures, before any very definite conclusions can be drawn as to the energy relations concerned in the fixation of nitrogen by *Azotobacter*.

SUMMARY

The experiments reported in this paper have been carried out with the object of determining the relative ability of different cultures of *Azotobacter* to utilize qualitatively and quantitatively calcium salts of the different fatty acids up to and including six carbon atoms. Two cultures have been studied intensively, others to a less extent. The quantity of acid and total nitrogen present in the medium before and after varying periods of incubation were determined. The rapidity of growth was recorded,

as was also qualitative tests of changes in hydrogen-ion concentration.

The following is a summary of the more important tentative conclusions indicated by the limited experimental data secured:

(a) The various strains of *Azotobacter* behave quite differently with respect to their ability to utilize different fatty acids. Some cultures have been tested that seem rather limited in this respect, while others may utilize all of the acids tested.

(b) Individual cultures may vary widely not only in their ability qualitatively to utilize different acids but there may also be a marked difference quantitatively in this respect.

(c) The iso compounds are not as readily metabolized as are the normal.

(d) There is a marked tendency toward the reduction of the hydrogen-ion concentration in a medium in which an acid is utilized by *Azotobacter*. In some instances, apparently, this phenomenon may be responsible not only for the cessation of growth but actually for the death of the organisms.

(e) The quantity of nitrogen fixed is more or less proportional to the quantity of acid utilized.

(f) The quantity of nitrogen fixed per unit weight of acid consumed increases as the molecular weight or heat of combustion increases, provided comparisons are limited either to normal or iso compounds. There is some indication that the efficiency with which *Azotobacter* can utilize various acids, as measured by the quantity of nitrogen fixed, increases as the molecular weight increases, even when the comparison is based upon the energy content of the material utilized.

(g) The cation with which the acid is combined apparently plays a very important role in determining the ability of an organism to utilize the acid.

(h) The quantity of nitrogen fixed when various acids are utilized is more closely correlated with the energy content than it is with the actual weight of the material consumed.

ACKNOWLEDGEMENT

I wish to take this means of expressing my appreciation to the officials of The Missouri Botanical Garden and The Kansas

Agricultural Experiment Station for the valuable aid rendered in making possible this investigation; and especially to Dr. B. M. Duggar under whose guidance the work was undertaken and whose constructive criticism has proved of inestimable value in its prosecution.

LITERATURE CITED

- Bonazzi, A. ('26). The mineralization of atmospheric nitrogen by biological means. IV Conf. Internat. de Pedologie, Actes 3: 74-115. 1926.
- Hoffmann, C., and Hammer, B. W. ('10). Some factors concerned in the fixation of nitrogen by *Azotobacter*. Wis. Agr. Exp. Sta. Res. Bul. 12: 152-172. 1910.
- Hunter, O. W. ('23). Stimulating the growth of *Azotobacter* by aeration. Jour. Agr. Res. 23: 665-677. 1923.
- Johnson, H. W., and Lipman, C. B. ('22). The effect of reaction on the fixation of nitrogen by *Azotobacter*. Univ. Cal. Publ. Agr. Sci. 4: 397-405. 1922.
- Krainsky, A. ('08). *Azotobacter chroococcum* und seine Wirkung im Boden. Centralbl. f. Bakt. II. 20: 725-736. 1908.
- Latahaw, W. L. ('16). Sodium sulfate as a substitute for potassium sulfate in the Gunning modification for determining nitrogen. Jour. Ind. and Eng. Chem. 8: 586. 1916.
- Löhnis, F., and Smith, N. R. ('16). Life cycles of the bacteria. Jour. Agr. Res. 6: 675-702. 1916.
- Mockeridge, Florence A. ('15). Soil organic matter as a culture medium for *Azotobacter*. Biochem. Jour. 9: 272-283. 1915.
- Shaffer, P. A., and Hartmann, A. F. ('21). The iodometric determination of copper and its use in sugar analysis. II. Methods for the determination of reducing sugars in blood, urine, milk, and other solutions. Jour. Biol. Chem. 45: 365-390. 1920-1921.

THE EFFECT OF ULTRA-VIOLET RADIATION UPON HIGHER PLANTS¹

ETHEL TABER ELTINGE

Jessie R. Barr Research Fellow in the Henry Shaw School of Botany of Washington University

INTRODUCTION

The sterilizing action of ultra-violet radiation has been known for over fifty years. Downs and Blunt in 1877, working with putrefying material, were the first to discover it. Since then there have been many workers in this field, and to-day ultra-violet sterilization is a more or less common practice.

At present, work with ultra-violet rays is carried on along two different lines. One line deals with the effects produced in higher animals and man with particular reference to the depth of penetration of the rays and to the changes produced within individual cells. The other line deals with the effects produced in plants. Little work was done in the latter subject until 1911 when Kluyver studied the effect on plants of a long-continued raying with an ultra-violet lamp. From then until 1918 the subject received little attention. Since then, however, it has taken on a fresh impetus, and to-day there are many people working in that field. At the present time it is generally known that raying with an unscreened quartz mercury lamp causes injury due to the presence of the short rays. The important line of research now is to determine the effects of the longer ultra-violet rays on the different groups of plants, and this can be done only by the use of specific screens to eliminate certain rays.

Under favorable conditions the spectrum of sunlight contains rays as short as $291\ \mu\mu$. Thus if a mercury vapor lamp is screened to absorb all rays shorter than $291\ \mu\mu$, the same type of rays penetrate as are found in sunlight, the only difference being that

¹ An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University and submitted as a thesis in partial fulfilment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany.

the ultra-violet rays are much more intense, since the atmosphere screens out much of this group of rays originally in sunlight.

HISTORY

THE EFFECT OF ULTRA-VIOLET RADIATION ON LOWER ORGANISMS

Many articles have been written on the effect of ultra-violet rays on bacteria. Potthoff ('20) found that a suspension of bacteria $3\frac{1}{2}$ mm. thick placed $15\frac{1}{2}$ cm. from the light gave the following results.

LENGTH OF TIME NECESSARY FOR THE DESTRUCTION OF BACTERIA

	Spores	Vegetative cells
<i>B. anthracis</i>	5 minutes	15 seconds
<i>B. subtilis</i>	9 minutes	2 minutes
<i>B. mesentericus</i>	5 minutes	1 minute

In pigmented forms a short exposure inhibited the production of pigment, but upon repeated short exposures the pigment again appeared.

Mashimo ('19) found that the rays most effective in the destruction of bacteria were those between 295 and 186μ . He proved this by using in a quartz spectrograph a culture of bacteria instead of a photographic plate and noticing the region where no growth appeared.

Bazzoni ('14) found that the destructive power of ultra-violet radiation in relation to bacteria increased rapidly with a decrease in wave length, but that this effect was in some way dependent upon association with longer wave lengths. Wave lengths of from 220 to 225μ killed the bacteria after several hours, while the same intensity of light containing full radiation destroyed them very rapidly.

Burge ('17) has proved that ultra-violet rays kill living cells such as bacteria, not by destroying the intra-cellular enzymes but by coagulating the protoplasm. For his work he used bacteria that liquefy gelatin and found that the organisms killed by ultra-violet when ground with sand produced as much liquefaction as ground living organisms.

Green ('97) found that the destruction of diastase in a leaf was less than in an extract of malt or saliva and concluded that

either the chlorophyll or the proteins of the protoplasm must act as a screen absorbing the injurious rays.

Tanner and Ryder ('23) have found that yeast cells are almost as susceptible to ultra-violet radiation as bacteria, although pigmented yeasts are more resistant than white ones.

Nadson and Philippov ('28) used a Bach model of a quartz mercury vapor lamp giving rays as short as $220\text{ }\mu$. Twenty-four-hour cultures of *Saccharomyces* and *Mucor* of different species on nutrient agar were rayed at thirty cm. from the light for ten to twenty minutes. For raying, the cover of the petri dish was removed and replaced by a piece of heavy glass with a circular opening in the center. After several days the region where there was no glass was devoid of growth, but just at the edge of the opening where only the slanting ultra-violet rays and hence the long ones were received, there was a marked increase in growth. Growth under the glass was normal. With yeast, not only increased, but abnormal, budding was noticed in the region of increased growth. With some fungi, asexual reproduction was increased while with others it was the sexual.

Larger organisms have been used for determining the effect of ultra-violet radiation on individual cells. Barr and Bovie ('23) used amoebae that had been cleared through starvation. They found that after an exposure of three-fourths of a second the amoebae ceased to move and after an exposure of one minute they were killed. At first the edge of the organism was irregular, but in a few seconds it became smooth by swelling. If irradiated for three to four minutes the animal swelled and clear spaces appeared between masses of protoplasm. Soon, however, crenulations were present about the border of the organism, giving the appearance of a loss of solution from the inside.

Tshuhotine ('23) thinks the rays first affect the plasma membrane, increasing permeability by coagulating the colloids. Then the surrounding medium enters and precipitates the protein colloids in the cytoplasm which surrounds the colloid particles of lecithin. Soon the base, coline, is formed which increases decomposition, giving OH ions which promote imbibitional swelling of the protein colloids of the cytoplasm until the cell is completely decomposed.

Brooks ('26) found that the shorter the rays, the greater the amount of 2,6-dibromo phenol indophenol penetrating cells of *Valonia*.

THE EFFECT OF ULTRA-VIOLET RAYS UPON HIGHER PLANTS

Bailey ('94) was the first to notice the harmful effect of light on plants. He used an electric arc light and found that if a piece of glass were placed between the light and the plant the injurious effects were modified. He found lettuce and radishes very sensitive to the arc light. A few hours raying caused leaves of *Coleus* to become shiny and lose their purple color when that color was present only in the upper epidermis. When cross-sections of the leaves were examined, the epidermis was found to be collapsed and opaque, coloring the leaf brown. Professor Rowlee, working with Bailey, concluded that the palisade tissue absorbs a large amount of water from the epidermis, due to greater protoplasmic activity, and the epidermis thus emptied collapses.

The next person to do any extensive work on plants was Kluyver ('11), who, using a quartz mercury lamp giving rays of 230μ and shorter, gave the plants one long exposure. He verified Bailey's results as to the injury produced in higher plants and its modification by using a screen of thick glass. Only the epidermis of leaves was found to be affected, but in roots and stems the injury was deeper. Anthocyanin was again found to be decomposed by the short rays which do not penetrate. The longer rays were found to have no effect on anthocyanin.

Ursprung and Blum ('17) used a new method for determining injury. After raying plants the desired time the cells were plasmolyzed in sugar solution and then deplasmolyzed if possible in clear water. The less the injury the greater was the per cent deplasmolyzed in water. Epidermis and cuticle were found to exert a little protection. Usually cells containing chlorophyll were more resistant than those lacking it. Diatoms were found very susceptible, due to the large amount of silica in their walls.

Stoklasa ('11) found that a long exposure to ultra-violet radiation injured the epidermal cells but did not harm the chlorophyll in adjacent cells. Etiolated seedlings turned green in two hours

upon exposure to rays of from 400 to 300 $\mu\mu$, while it took six hours to produce the same result in sunlight.

Schanz ('20) found that when the rays below 320 $\mu\mu$ were cut off the larger part of the red color disappeared from red-leaved lettuce. In like manner he caused the leaves of copper beech to become green.

Sheard and Higgins ('27) reported the effect of ultra-violet radiation on germination and growth of seeds. They used an unscreened quartz mercury lamp and screens of ultra glass, vita glass, and ordinary glass. In general they found that wave lengths of 270–320 $\mu\mu$ delayed the time and lessened the rate of growth, probably because of changes which carried to their extreme eventuate in the coagulation of the seed albumin. Rays of 320–390 $\mu\mu$ were particularly effective in promoting growth. When seedlings of lettuce, radish, and turnip were irradiated one, two, five, and ten minutes, those which normally germinate and grow in darkness showed most rapid germination and best growth when not rayed. Minimum growth was found in seedlings grown in diffused light. Radiation of these seedlings for two to three minutes by a quartz lamp accelerated the germination and subsequent growth as compared with non-rayed seedlings under similar conditions. Thus they state that raying with the near ultra-violet region aids germination and growth of a cell or normal functioning of an organism which is kept under unphysiologic environment.

Russell and Russell ('27), using a Hewittic mercury vapor lamp, found that when etiolated mustard seedlings were given short daily exposures to ultra-violet rays, dwarfing resulted in direct proportion to the length of exposure. Some chlorophyll appeared in all rayed seedlings. In seedlings grown under normal daylight conditions the dwarfing was not as great.

Dane ('27) found that soybeans irradiated by ultra-violet rays were dwarfed and the leaf and stem tissue brittle and stiff. Stems of irradiated plants were $1\frac{1}{2}$ times as great in diameter as those of control plants. Rayed stems were hollow and showed reduction in medullary rays, the meristematic tissue thus remaining active for a much longer time than that in control plants. The ordinary parenchymatous cells of the medullary rays had developed into xylem and phloem.

Beeskow ('27) found that a daily irradiation of more than $\frac{1}{2}$ minute caused injury to soybeans, but that irradiation of $\frac{1}{2}$ minute caused no injury and might stimulate growth. When corn plants were rayed they showed an increased calcium and phosphorus content.

McCrea ('27) grew *Digitalis purpurea* to the ten-leaf stage in a greenhouse glassed with vita glass. She found greater growth and darker color than in control plants. The plants were then put outdoors and when cuttings were taken in August and September, the rayed plants were found to contain an increased amount of digitalin.

Delf and Ritson (Delf, Ritson, and Westbrook, '27) irradiated *Pelargonium*, *Coleus*, *Fuchsia*, *Abutilon*, *Salvia*, and *Trifolium* for various lengths of time and found retarded growth, delayed germination, retarded flower formation, and leaf fall. In addition there was a loss of anthocyanin by *Coleus* and in many cases a deeper green color produced in *Coleus* and other plants. Six-weeks-old seedlings of *Trifolium* when rayed one-half minute daily showed increased growth.

Westbrook (Delf, Ritson, and Westbrook, '27) used different lengths of days in addition to short exposures to ultra-violet radiation. In all cases injury was greater the shorter the day. The injury consisted in the development of thinner leaves with more compact mesophyll and smaller and fewer air-spaces, reduction of mechanical tissue, and collapse of the cells of the upper epidermis followed by a withdrawal of the chloroplastids from the upper ends of the palisade cells.

Tsuji ('18) obtained increased growth and a higher percentage of sugar in sugar cane grown in sunlight and rayed daily with a weak ultra-violet lamp. When pineapples were grown in sunlight plus a daily raying of forty minutes, the pineapples were sweeter, juicier, and larger than normal. When banana leaves and stalks were exposed to ultra-violet rays after being cut they kept fresh longer than similar leaves and stalks not rayed.

Clement ('26) has found that apples rayed for three hours showed a slight yellowing of the green side, and that when these apples were stored the rayed sides did not regain their green color but remained turgid longer than those not rayed.

Nadson and Rochline-Gleichgewicht ('28), using a Bach model of an ultra-violet lamp emitting rays down to $220\text{ }\mu$, have found that ultra-violet rays cause crystals of calcium oxalate to form in the cells of *Elodea densa*, *Elodea canadensis*, and *Pterygophyllum hepaticaeifolium*. These plants were barely covered with water and placed thirty cm. away from the lamp for ten to thirty minutes. The crystals began as small granules, with a chloroplast often as the center, and increased to good size. After two to four days they dissolved simultaneously with the death of the cell. If the cells were treated with a narcotic before raying no crystals were formed.

THE EFFECT OF ULTRA-VIOLET RADIATION UPON ORGANIC MATERIALS

Calabek ('27) determined the effect of ultra-violet rays upon the swelling of biocolloids such as agar. When agar discs were rayed a marked decrease in swelling resulted. It was found that the effect of raying could be preserved in dry agar for several months even if the agar were redissolved. As a result the hypothesis was advanced that the effect of ultra-violet rays upon plants is due to a lowering of the swelling capacity of protoplasm and cell wall in the upper cellular layers of the plant.

Hess ('26) and others have found that when foods are rayed they are rendered rickets-protective. In vegetable foods phytosterol is activated while in animal foods it is the cholesterol that is acted upon.

THE PENETRATION OF ULTRA-VIOLET RAYS

Several authors including Henri ('12) have found that the depth of penetration of the shorter ultra-violet rays through the skin is not more than .1 mm. However, for this work dead skin was used.

Macht, Anderson, and Bell ('28), using living anesthetized animals, have found that with an exposure of one minute ultra-violet rays as short as $302\text{ }\mu$ penetrate through living skin that is more than .1 mm. in thickness. When an exposure of two minutes was given rays as short as $280\text{ }\mu$ passed through. They then tested the penetration of rays into the peritoneal cavity of

rabbits and found that with an exposure of two minutes, rays as short as $313\ \mu$ penetrated into the cavity. Next they compared the penetration of living skin with that of dead skin and found the former much more penetrable. When the dead skin was treated with a lipoid solvent it became as transparent to ultra-violet rays as living skin. The pigment in the skin of a negro was found to absorb almost all the short rays. The same result was obtained by injecting a rabbit intravenously with 1 per cent eosin solution.

STATEMENT OF THE PROBLEM

The problem in this paper is to determine the effect of ultra-violet light as a whole on higher plants, and whether the longer ultra-violet rays stimulate growth in higher plants.

MATERIALS AND METHODS

EXPERIMENTAL

The lamp used for this work was an air-cooled Uviarc quartz lamp from the Burdick Cabinet Co. In the experiments designated as Series I this lamp was used without a screen of any kind. When used in this way the rays given off range from 578 to $200\ \mu$ (5780 A. U.-2000 A. U.) (fig. 1).

When a screen of vita glass from the Hires Turner Glass Co. was interposed between the light and the plants the rays had a range of 578-289 μ (5780 A.U.-2894 A.U.). The experiments using this screen constituted Series II.

A screen of quartz-lite glass from the American Window Glass Co. interposed between the light and the plant permits the passage of rays ranging from 578 to $313\ \mu$ (5780 A.U.-3136 A.U.). Experiments using this glass are described in Series III.

The ultra-violet rays produced by a quartz mercury lamp may be divided into two groups, first, the abiotic rays (short rays), with wave lengths ranging from 185 to $290\ \mu$, which are reducing rays and hence killing rays, second, the biological rays (long rays) which range from 290 to $400\ \mu$. These are oxidizing rays and hence stimulating. The abiotic rays being very readily absorbed by the atmosphere are never present in sunlight when it reaches the earth, and were essentially eliminated where either of the

glass screens was used. The lamp was used at 50 and at 100 inches from the plant both with and without screens.

Although the atmosphere between the lamp and the plant absorbs some of the short rays, the distance of 100 inches is not sufficient to absorb all the short rays, and 50 inches without a screen allows a large percentage of the short rays to reach the plants. When a lamp screened by vita glass is used at a distance of 50 inches from the plants most of the short rays are absorbed, but none of the long ones. The same screen used at 100 inches

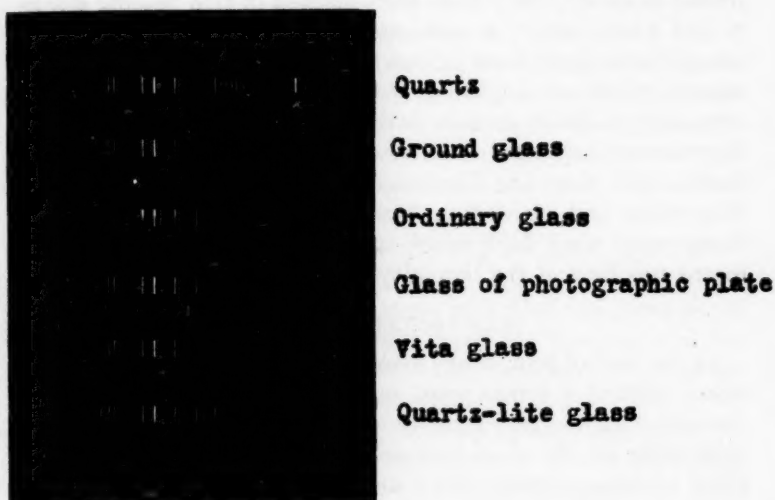


Fig. 1. Showing the spectrum of the different glasses.

from the plants allows only a large percentage of the long rays to reach the plants. When a screen of quartz-lite glass is used instead of vita glass, plants at a distance of 50 inches receive all the long rays and no short ones. The same screen at 100 inches allows only a part of the long rays to reach the plants.

Except for one group of experiments mentioned later the exposure began with 30 seconds the first day and each day was increased that amount. All plants under experiment were moved about each week in the greenhouse to eliminate all differences in environmental conditions.

The plants used were as follows: *Lactuca sativa* L. var. "Black-seeded Forcing"; *Raphanus sativus* L. var. "Early Scarlet Turnip White Tip"; *Cucumis sativus* L. var. "Improved Green Hybrid"; *Ipomoea Batatas* Poir.; *Phaseolus vulgaris* L. var. "Stringless Green Pod"; *Nicotiana Tabacum* L.; *Coleus Blumei* Benth. var. *Verschaffeltii* Lem. and vars. "Spotted Gem," "Defiance" and "Trailing Queen"; *Bryophyllum pinnatum* Kurz.; *Zea Mays* L. var. "Stowell's Evergreen."

Cuttings of *Ipomoea*, *Coleus*, and *Bryophyllum* were made and rooted in sand. They were then planted in rich potting soil in 3- and 4-inch pots. As soon as the plants had recovered from transplanting they were put under the conditions of the experiments. Seeds of *Raphanus*, *Lactuca*, *Cucumis*, and *Nicotiana* were sown in flats. As soon as the plants appeared above ground they were transplanted to 3-inch pots and put under experiment. Seeds of *Zea Mays* and *Phaseolus* were germinated between moist filter-paper and planted in 3-inch pots. All plants when not being rayed were kept under usual greenhouse conditions. A record was kept of the humidity and temperature.

ANATOMICAL METHODS

At the end of four weeks samples of the leaves of the plants rayed without a screen were taken for anatomical study. At the end of eight weeks samples of leaves and stems of all plants were taken for the same purpose. Care was taken in the sampling to take portions which were in corresponding positions on the plants and otherwise as nearly equivalent as possible.

The leaves and smaller stems were killed in medium chromo-acetic killing fluid and imbedded in paraffin. The larger stems were cut free-hand while fresh. Two stains were used: Haidenhain's Iron Haematoxylin and Safranin-Delafield's Haematoxylin.

PHYSIOLOGICAL METHODS

The rate of chlorophyll decomposition under the different screens, in sunlight, and in diffused light was tested, using an 80 per cent alcoholic solution of chlorophyll in vitreosil test-tubes.

The P_H of rayed and control plants in Series I was determined by the colorimetric method. The plant material was pressed in

a mortar and filtered through cotton. Then after diluting 1 to 10 with distilled water the chlorophyll was removed by filtration through an atmometer cup. The indicator was then added to the diluted juice freed from chlorophyll and the result compared with standard tubes.

Starch Storage.—Stem sections were made from fresh material of *Coleus* and *Phaseolus* in the different groups. These were stained with a standard iodine solution to show the distribution of starch.

Dry Weights.—For determination of dry weights plants were dried to constant weight in an oven run at 110° C.

Ash Determination.—Three grams of dry leaf material of the different plants were put into weighed crucibles and burned over a bunsen burner until a large part of the carbon had disappeared. To finish the burning, the crucibles were put into an electric oven at 600° C.

EXPERIMENTAL OBSERVATIONS

SERIES I

For all varieties of plants this series is divided into three parts, group H, which includes the plants rayed at 50 inches from the light; group F, those rayed at 100 inches; and as controls, group G, the same number of plants not rayed.

Series I H (rayed at 50 inches from the light without a screen).—Six young seedlings of *Cucumis* were rayed. The first evidence of the effect of ultra-violet rays appeared on the eighth day, when a slightly shiny appearance of the upper epidermis was noted. By the twelfth day there was evident curling of the edges of the leaf. At the end of three weeks the rolling of the leaves was very evident and the lower ones were turning brown and dying. The young leaves never attained as large a size as those on the control plants. By the end of the twenty-ninth day, when the plants received an exposure of fourteen minutes, one to three flowers were present, but the younger leaves were so rolled that the upper surface was hardly visible. When samples of the leaves were taken at the end of four weeks for anatomical study, they were found to be very brittle. The plant as a whole was very stiff and erect.

The control plants (G) had slightly more leaves and were a little taller. They also had several more flowers. Not only were the leaves greater in number but greater in size, being twice that of the rayed leaves. No rolling of the leaves was noted in the control plants. The color of the leaves was the same in both rayed and control plants except in the older rayed leaves which were brownish.

Six *Ipomoea* plants were used. By the sixth day the younger leaves showed a slightly blistered appearance which increased as time went on. By the eleventh day the veins were brown and the larger leaves showed several brown spots which seemed to be more or less superficial. By the end of three weeks the edges of the leaves had turned down. Very few leaves were shed. At the end of four weeks these leaves were also found to be brittle. The control plants again showed larger and more numerous leaves with no browning.

In the six *Nicotiana* plants, the first effect of raying was noticed on the eleventh day, when the margin of the leaves appeared wavy. By the thirteenth day the edges were definitely rolling upward. About the same time the upper surface became very shiny, and the leaves were so brittle it was almost impossible not to crack them. At the end of three weeks the older leaves were turning yellow and the younger leaves were so rolled that the upper surface was hardly visible, though no leaves were shed. The control plants showed none of these characteristics, the upper surface of the leaves being very hairy and the leaves larger.

The four varieties of *Coleus Blumei* were put into two groups according to their resistance to ultra-violet radiation. The group most sensitive to ultra-violet contained vars. *Verschaffeltii* and "Spotted Gem." At the end of five days a fading of the red color was noticed, and at the end of ten days practically all the red color had disappeared. The glossy upper surface was broken only by the bases of the hairs appearing as dots. The two halves of the leaves were rolled upward toward the midrib and the tips downward, so that the leaves appeared to be only half their normal size and were very brittle (pl. 22, fig. 4). By the end of four weeks all the older leaves had fallen and only a few of the

younger remained and those were very small and abnormal in shape.

The other group containing vars. "Trailing Queen" and "Defiance" seemed to be a little more resistant to ultra-violet rays. Here the first indication of a loss of red color appeared the eighth day. About the same time the shiny dotted appearance of the upper surface of the leaves was observed. The same rolling of the leaves was noted as in the other group. "Trailing Queen" lost very few leaves even at the end of four weeks but var. "Defiance" began to shed its leaves at the end of three weeks.

In the varieties of *Coleus* where any red color was present in the stem a loss of it began to be noted in the tip of the stem at the end of five days and a complete loss at the end of ten days. If after a raying of four weeks these plants were put back under normal greenhouse conditions the red color appeared again to a certain extent in the decolorized tip of the stem and the new growth of stem and leaves was normal.

Ten very young seedlings of *Raphanus* were rayed. At the end of eight days the typical curling upward and shiny appearance of the leaves was noted. Here the rayed leaves seemed to be a little deeper green than the control leaves. At the end of four weeks the leaves and petioles were almost as brittle as the rayed *Nicotiana* leaves. At the end of eight weeks the plants were so curled they appeared almost dead. The roots of the control plants, as well as the leaves, were much larger, as will be seen in pl. 21, fig. 7.

Two sets of *Lactuca*, containing ten plants each, were used, one set having two leaves and the other nine to ten. The object was to see if the older plants would be more resistant to ultra-violet radiation. The set of plants having two leaves never seemed to get much larger, as will be seen in pl. 22, figs. 2 and 3. At the end of two weeks the leaves were noticeably smaller and fewer than on control plants. All the new ones formed were abnormal and the older ones soon dried up and dropped off. As time went on the difference between the rayed and control plants became the most striking of any of the plants tried. At the end of eight weeks the rayed plants had an average of 4.25 leaves per plant while their controls had 13.

This and one set of *Raphanus* plants were the only groups of plants under Series I H that were rayed for eight weeks, the others being discontinued at the end of four weeks. A comparison of these plants at the end of four weeks and eight weeks with similar plants rayed at 100 inches (F) will be seen in pl. 22, figs. 2 and 3.

The set of plants having nine to ten leaves was found to be a little more resistant. At the end of one week the oldest leaves began to show tiny brown spots scattered over their surfaces. Soon after they began to dry up. At the end of three weeks all the older leaves were dead and the new ones smaller but not nearly as small as the new ones in the group having two leaves at the beginning. The leaves were very brittle and even the youngest very curly and brownish (pl. 22, fig. 1). At the end of eight weeks the rayed plants had an average of 18.32 leaves per plant and the controls 25.8 leaves (table I).

TABLE I

SHOWING RATE OF GROWTH IN LACTUCA. FIGURES INDICATE THE AVERAGE NUMBER OF LEAVES PER PLANT

Date	Series number							
	I H		I G		I H		I G	
	Pres.	Lost	Pres.	Lost	Pres.	Lost	Pres.	Lost
Mar. 1	2.00	0	2.00	0	10.00	0	10.00	0
Mar. 8	3.60	0	3.40	0	10.60	2.00	11.60	1.30
Mar. 15	4.60	.60	4.60	0	10.40	4.50	12.20	2.60
Mar. 22	4.00	2.30	6.20	0	10.20	4.40	11.30	4.10
Mar. 29	4.30	3.10	7.00	1.50	12.60	2.10	14.30	1.33
Apr. 5	5.10	.30	8.00	.90	15.10	2.40	18.16	1.00
Apr. 12	5.00	1.10	10.00	1.20	15.66	5.33	21.20	3.90
Apr. 19	4.80	1.20	10.20	2.40	18.33	4.00	25.80	3.90
Apr. 26	4.25	5.00	13.00	1.00				
Net total	2.25	13.60	11.00	7.00	8.33	24.73	15.80	18.13

In *Bryophyllum* the first evidence of raying appeared the sixth day in the form of a glossy upper surface. At the end of two weeks the new leaves were very abnormal in form, the halves rolling upward from the midrib but the leaf itself not curling.

Three sets of *Phaseolus* seedlings were used with six plants in each. One set had both cotyledons intact, another had one

cotyledon removed, and the third had both cotyledons removed. The object was to determine if the removal of stored food had any influence on the effect of raying. In all cases growth was retarded and burning resulted. The leaves became very blistered and abnormal in shape. The difference between rayed and control plants with both cotyledons removed was very great but the difference with one cotyledon removed was about the same as where both cotyledons were intact (table v).

Series I F (rayed at 100 inches from the light without a screen).—The results with *Cucumis* here were in general the same, though never as marked for the same amount of raying, as in Series I H. The appearance of injury was retarded several days, being noted first on the fifteenth day when the plants received an exposure of $7\frac{1}{2}$ minutes. For comparison of the size of rayed and control plants see table II and pl. 21, figs. 1 and 2. At the end of four weeks the average dry weight of rayed plants was 0.616 grams and the control plants 1.29 grams. At the end of eight weeks the leaves were as rolled as in Series I H.

TABLE II

SHOWING RATE OF GROWTH IN CUCUMIS. FIGURES EXPRESS AVERAGES PER PLANT

Date	Series number					
	I F			I G		
	Leaves pres.	Leaves lost	Ht. in cm.	Leaves pres.	Leaves lost	Ht. in cm.
Feb. 1	2.00	0	0	2.00	0	0
Feb. 8	6.00	0	7.35	5.25	0	6.00
Feb. 15	7.00	.75	9.55	7.00	1.25	7.70
Feb. 22	7.50	.20	11.60	8.00	.20	11.25
Feb. 29	9.00	.30	13.80	10.00	.28	14.00
Net total	7.00	1.25	13.80	8.00	1.73	14.00

The experiment using *Ipomoea* plants was continued for eight weeks. A slight browning of the veins was noticed at the end of the eighteenth day when the plants received an exposure of nine minutes. At the end of four weeks the usual blistering appeared as can be seen in pl. 21, fig. 6. About as many leaves were added as to the control plants but they never attained as large a size.

Nicotiana behaved very much the same here as in Series I H except that the effects were a little later in appearing.

The same four varieties of *Coleus Blumei* were used as in the preceding series and were again divided into two groups. The effect on both groups was somewhat less marked and much retarded, particularly as far as shedding leaves was concerned. The more resistant group shed no leaves until the fifth week, and at the end of seven weeks only a few had been shed (pl. 23, figs. 1-4). When these plants were put under normal greenhouse conditions at the end of eight weeks raying, they began to show normal growth and color after ten days, but at the end of four weeks they were still far behind the control plants (pl. 22, fig. 5).

Two sets of *Raphanus* were used, one with two leaves and the other with four to five leaves. Here there seemed to be very little difference in the effect produced whether the plant was just above ground or had several leaves. The effect of raying did not appear until the eighteenth day, but at the end of four weeks it was quite marked (pl. 21, fig. 5). At the end of eight weeks there was a noticeable difference in the number of leaves, the rayed plants averaging 6.2 and the control 8.42 leaves per plant (table III (a), and pl. 21, fig. 4).

The same two sets of *Lactuca* plants were again used. The same general results were found for the set having two leaves, but in the set having nine leaves the rayed plants produced as well as lost more leaves than the controls though they were never as large. A comparison of the effects produced here with those in Series I H can be well seen in pl. 22, figs. 1, 2, and 3 and table III (b). In addition to the above two sets of plants two more sets were used, one set consisting of two-leaved lettuce plants of a red variety and the other of old lettuce plants with fifteen leaves. Red lettuce was rayed to see if the color would disappear as it had done in *Coleus*. However, after raying for eight weeks the red color was still evident though partly masked by the brownish effect of raying. The old *Lactuca* plants were used in order to determine the effect of ultra-violet light on bud and flower formation. The same retarding effect was found though less with these older plants. At the end of eight weeks both rayed and control plants were

budded. The flower stalks of the control plants were greater in diameter and seemed to branch more at the top (pl. 26, fig.1).

Bryophyllum rayed under these conditions showed no curling of the leaves, due no doubt to their thickness. However, the leaves again rolled upward from the midrib. At the end of two weeks they had shiny brownish surfaces similar to those found in many of the other plants, and often parts of the new leaves formed were undeveloped. For a comparison of the rate of growth in rayed and control plants see table III (c) and pl. 26, fig. 5.

The ten *Zea Mays* seedlings were found to be as resistant to ultra-violet light as any of the plants used, showing the first evidence of any harmful effect the twenty-fourth day when they received a twelve-minute exposure. Even then the effect was slight, taking the form of a slight rolling upward of the edges of the leaves. When the rate of growth was compared, the rayed

TABLE III

SHOWING RATE OF GROWTH IN SERIES I F AND G. FIGURES INDICATE THE AVERAGES PER PLANT

Date	(a) <i>Raphanus</i>				(b) <i>Lactuca</i>							
	I F		I G		I F		I G		I F		I G	
	Pres.	Lost	Pres.	Lost	Pres.	Lost	Pres.	Lost	Pres.	Lost	Pres.	Lost
Mar. 1	2.00	0	2.00	0	2.00	0	2.00	0	9.0	0	9.0	0
Mar. 8	4.00	0	3.91	0	3.75	0	3.7	0	12.0	1.50	10.4	2.2
Mar. 15	5.63	0	5.63	0	4.28	0	4.2	0	12.9	2.70	10.6	2.1
Mar. 22	6.90	0.09	6.16	0.03	5.21	1.7	7.0	0	12.9	3.65	10.75	2.3
Mar. 29	6.90	1.00	6.53	0.03	6.07	3.2	7.4	1.7	10.35	5.80	9.6	4.2
Apr. 5	6.60	1.60	6.64	1.06	6.85	1.4	9.2	0.7	12.6	1.80	12.4	1.5
Apr. 12	7.20	1.00	7.71	0.10	8.80	1.7	11.8	0.4	15.5	2.50	15.5	.7
Apr. 19	6.20	1.60	8.42	0.18	8.77	3.3	12.6	2.2	19.0	3.66	18.4	3.5
Apr. 25					11.55	1.6	16.4	1.8	22.1	1.40	20.6	1.5
Net total	4.20	5.29	6.42	1.40	9.55	12.9	14.4	6.8	13.1	23.01	11.6	16.02

Date	(c) <i>Bryophyllum</i>				(d) <i>Zea Mays</i>			
	I F		I G		I F		I G	
	Lvs. pres.	Ht. in cm.	Lvs. pres.	Ht. in cm.	Lvs. pres.	Ht. in cm.	Lvs. pres.	Ht. in cm.
Jan. 24	12.59	13.14	13.71	14.35
Feb. 1	13.71	13.85	14.28	15.71	3.25	3.31	3.50	3.62
Feb. 8	14.85	15.14	15.71	17.35	3.75	5.2	4.00	5.37
Feb. 15	15.14	16.21	16.85	18.57	4.50	6.37	5.00	8.00
Feb. 22	17.00	17.04	17.42	19.07	5.00	8.43	6.25	9.94
Feb. 29					6.00	10.60	6.90	14.20
Net total	4.41	3.90	3.71	4.72	6.00	10.60	6.90	14.20

plants showed a noticeable retardation (table III (d) and pl. 26, fig. 4).

SERIES II

Plants in this series were under exactly the same conditions as those in Series I except that here a screen of vita glass was used. The series was again divided into three parts, group A, rayed at 50 inches from the light, group B, rayed at 100 inches, and group E, which was the control for both this series and Series III. All plants rayed were treated daily for seven weeks.

Series II A (rayed at 50 inches from the light with a screen of vita glass).—Plants rayed under these conditions responded very differently.

In ten young *Cucumis* seedlings exposed to ultra-violet leaves were produced a little more rapidly than in the control plants (E) but at the end of seven weeks the control plants had slightly more leaves than the rayed ones. The size and color seemed to be about the same for both. The stems elongated almost equally for the first three weeks and then increased very rapidly in the rayed plants, so that at the end of seven weeks they averaged eight to nine centimeters longer and were noticeably greater in diameter than the control plants (table IV (a) and pl. 24, fig. 1). Flowers appeared on the rayed plants two days earlier than on the control plants. At the end of seven weeks the rayed plants averaged 2.3 flowers and the control plants only 1.2 flowers per plant.

Ten cuttings of *Ipomoea* of as near the same size as possible were rayed. Even at the end of seven weeks there was very little difference in height between them and the controls, though the average number of leaves added was much greater in the rayed plants (table IV (b) and pl. 24, fig. 2). The leaves of both rayed and control plants were of about the same size and color.

Observations on fifteen *Nicotiana* plants point toward a retardation of growth, though there was no evidence of any burning. The leaves were about the same in number, size, and color. The stem, however, was taller and smaller in diameter in the control plants. No difference was noticed in the time of

flowering. In general, the rayed plants gave the appearance of being more stocky (table iv (c)).

Ten young *Zea Mays* seedlings were used. At first the control plants grew taller, measuring from the base to the highest node. During the last few days, however, the rayed plants grew very rapidly and surpassed the controls. The rayed stalks were also larger in diameter. The leaves on the rayed plants not only outnumbered those on the control plants, but were from 1.5 to 2.0 cm. wider in the middle, those on the controls averaging 4.0 cm. in width (table iv (d) and pl. 24, fig. 3).

From the very beginning the twenty rayed plants of *Lactuca* produced slightly more leaves than the controls. The leaves of both were the same in color, texture, and size (table iv (e) and pl. 24, fig. 4).

The size of the leaves on the ten rayed *Raphanus* seedlings equalled those on the controls, but there were slightly more leaves on the latter (table iv (f) and pl. 25, fig. 1).

Coleus Blumei vars. "Spotted Gem" and *Verschaffeltii* have been found to be the varieties most sensitive to ultra-violet light, and six cuttings of each as near the same size as possible were used. Both varieties showed the same characteristics. From the very beginning the rayed plants showed greater growth both as to number and size of leaves, and as to height and diameter of stem (table iv (h and i), and pl. 25, figs. 2 and 3). The red color did not seem to be affected as it was in Series I.

Eight cuttings of *Bryophyllum* were rayed. At first the controls grew taller, with a greater number of leaves, but during the last few weeks the rayed plants much surpassed the controls (table iv (g) and pl. 25, fig. 4).

Three sets of *Phaseolus* seedlings of six each were used as in Series I. Those with both cotyledons intact and with one cotyledon removed were taller and had more leaves than the corresponding control plants. Those with both cotyledons removed had slightly more leaves than the corresponding control plants, but were shorter (table v).

Series II B (rayed at 100 inches from the light using a screen of vita glass).—The same number of *Cucumis* plants was used as in Series I A. From the very first the number of leaves on the

rayed plants exceeded those on the controls, but the size of the leaves was about the same in both. The rayed leaves in this group seemed a little deeper green than did the controls. All through the experiment the stems of the rayed plants increased in both length and thickness more rapidly than those of the control plants, and at the end of seven weeks were a little greater in length than the stems of the plants in Series II A (table iv (a) and pl. 24, fig. 1).

About the same number of leaves was present on the ten *Ipomoea* cuttings in this series as in Series II A, which was much greater than in control plants. The stem, however, was greater in length here than in either Series II A or the control E (table iv (b) and pl. 24, fig. 2).

In the fifteen *Nicotiana* plants rayed little difference was found from Series II A either in number or color of leaves or in length and thickness of stem. The control plants had about the same number of leaves but were taller (table iv (c)).

In this group the ten *Zea Mays* seedlings showed a slight increase in number of leaves over those in Series II A and a greater increase over the control plants in E. In average growth in height and diameter of the stalk this group exceeded both the control plants in E and the rayed plants in A (table iv (d) and pl. 24, fig. 3).

Here, as in Series II A, twenty *Lactuca* plants were rayed. All through the experiment there was a slight increase in number of leaves over that in the control plants though the size of the leaves was a little greater in the control plants (table iv (e), and pl. 24, fig. 4).

The ten rayed *Raphanus* seedlings showed leaves equalling those of the control plants in size but fewer in number. The seedlings in this group were almost identical with those in Series II A (table iv (f), and pl. 25, fig. 1).

The same number of *Coleus* cuttings was used as in Series II A. As to number of leaves produced the plants in this group about equalled those in Series II A, but much surpassed the controls in E. Here the plants exceeded in height both those in Series II A and the controls E (table iv (h and i) and pl. 25, figs. 2 and 3).

The *Bryophyllum* cuttings in this group produced about the

same number of leaves as the controls but increased a little more in height than did the controls. Plants in Series II A surpassed this group both in number of leaves and in height (table iv (g) and pl. 25, fig. 4).

SERIES III

The same conditions were present in this series except that instead of vita glass a screen of quartz-lite glass was used. This series also was divided into three parts, group C, rayed at 50 inches from the light, group D, rayed at 100 inches, and group E, again the control. The same number of plants were used in each case as in Series II.

Series III C (plants rayed at 50 inches from the light using a screen of quartz-lite glass).—The number of leaves produced on rayed *Cucumis* seedlings was a little more than in Series II A, but a little less than in Series II B. As to growth in height both Series II A and B surpassed this group by about three centimeters. However, this group surpassed the control by more than five centimeters (table iv (a) and pl. 24, fig. 1).

The *Ipomoea* cuttings in this group produced more leaves than in either A or B of Series II, though the growth in height was a little less here than in those groups. This group showed better growth than the control in all respects (table iv (b) and pl. 24, fig. 2).

There was little difference between the growth of the *Nicotiana* plants here and in groups A and B of Series II. The control plants surpassed all three mentioned groups in height, but about equalled the other groups in number of leaves (table iv (c)).

The *Zea Mays* seedlings here were almost identical with those in Series II B as to number and size of leaves and as to height of plant. It would be hard to determine from external observation which of these two groups produced the better growth (table iv (d) and pl. 24, fig. 3).

The *Lactuca* plants in this group showed more leaves than either the control plants or the plants in Series II A and B. However, the leaves in this group of plants were a little smaller than in the control plants (table iv (e) and pl. 24, fig. 4).

The average number of leaves present in *Raphanus* seedlings in this group was slightly less than in the controls, but otherwise the plants were identical (table iv (f) and pl. 25, fig. 1).

Both varieties of *Coleus* again showed similar results. The cuttings in this group showed more growth in height and number of leaves than did the controls but not as much as in either A or B of Series II (table iv (h and i) and pl. 25, figs. 2 and 3).

The *Bryophyllum* cuttings in this group surpassed the control plants and those in Series II B both in number of leaves and in height. However, the growth in this group did not equal that in Series II A (table iv (g) and pl. 25, fig. 4).

As in Series II A, three sets of *Phaseolus* seedlings were used. Those with both cotyledons intact and with one cotyledon removed were a little taller and had slightly more leaves than the control plants, but were not quite as tall nor did they possess quite as many leaves as those in Series II A. The plants with both cotyledons removed showed less growth in height than the corresponding controls, but a little more than those in Series II A. The number of leaves present differed very little (table v).

Series III D (plants rayed 100 inches from the light using a screen of quartz-lite glass).—*Cucumis* seedlings in this group differed very little from those in group C and thus were several centimeters greater in length than the controls (table iv (a) and pl. 24, fig. 1).

Ipomoea plants showed greater growth in height in this series than in Series III C, with about the same number of leaves present in each series (table iv (b)).

The *Nicotiana* plants closely resembled those in Series II A and B, being stocky while the controls were much taller (table iv (c)).

Seedlings of *Zea Mays* in this group were not quite as tall as in the other groups mentioned, but nevertheless surpassed the control plants. As to number of leaves present this group about equalled the other groups. All the rayed groups in Series II and III, as previously mentioned, possessed leaves from one and a half to two centimeters wider in the middle than the control leaves (table iv (d) and pl. 24, fig. 3).

Lactuca plants in this group gave a slightly smaller count of leaves than in Series III C, but more than in Series II A and B and also more than the control plants. Little difference was noticed between the size of the leaves here and in the control plants (table iv (e), and pl. 24, fig. 4).

Raphanus plants showed slightly fewer leaves than in Series III C and thus fewer than the control plants (table iv (f) and pl. 25, fig. 1).

Both varieties of *Coleus* plants in this group surpassed the control both in number of leaves and growth in height, but showed fewer leaves and less growth in height than in Series II A and B and Series III C (table iv (h and i) and pl. 25, figs. 2 and 3).

Cuttings of *Bryophyllum* showed fewer leaves than in Series II A or Series III C and about the same number as the control plants and Series II B. However, this group showed almost as great growth in height as in Series III C, which surpassed Series II A and B and also the controls (table iv (g)).

TABLE IV

SHOWING THE RATE OF GROWTH OF PLANTS IN SERIES II AND III. FIGURES REPRESENT AVERAGE NUMBER OF LEAVES PER PLANT; AND HEIGHT IN CENTIMETERS

(a) Cucumis										
Date	Series II				Series III				Control	
	A		B		C		D		E	
	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.
Oct. 24	2.00		2.00		2.00		2.00		2.00	
Oct. 31	3.50	7.45	3.80	7.60	3.80	6.35	3.60	7.75	3.00	6.8
Nov. 7	5.00	8.90	5.00	10.00	4.90	8.30	5.00	9.85	4.80	7.9
Nov. 14	5.60	9.85	6.00	11.20	5.87	9.50	6.00	10.60	5.77	9.5
Nov. 21	7.10	13.00	6.70	14.00	6.77	11.33	6.80	13.05	5.90	11.1
Nov. 28	7.20	16.90	7.00	19.20	6.77	14.83	6.80	17.15	6.77	13.0
Dec. 5	7.10	22.70	8.11	24.72	7.66	20.00	6.80	22.25	7.44	17.6
Dec. 12	7.30	29.05	8.22	29.77	7.77	26.04	7.10	27.90	7.65	20.5
Net total	5.30	29.05	6.22	29.77	5.77	26.04	5.10	27.90	5.65	20.5

(b) Ipomoea										
Date	Series II				Series III				Control	
	A		B		C		D		E	
	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.
Oct. 24	11.12	11.25	7.25	12.82	9.12	8.44	8.25	8.56	5.87	5.75
Oct. 31	14.62	11.50	9.78	13.81	11.87	9.64	10.37	11.37	6.75	6.25
Nov. 7	16.75	12.62	11.75	14.81	13.62	10.59	13.00	12.25	8.50	6.94
Nov. 14	18.25	13.63	14.75	15.21	16.00	11.37	16.25	12.81	10.31	7.97
Nov. 21	20.70	14.50	18.00	16.57	19.87	12.75	17.62	14.06	10.75	8.43
Nov. 28	24.00	15.18	22.00	17.42	24.56	15.21	22.00	15.31	12.75	9.81
Dec. 5	26.75	15.68	25.71	19.57	27.42	16.50	26.06	15.42	14.42	10.35
Dec. 12	28.80	16.50	27.33	20.66	30.14	17.85	31.51	18.00	14.50	10.50
Net total	17.68	5.25	20.08	7.84	21.02	9.41	23.26	9.44	8.63	4.75

(c) *Nicotiana*

Date	Series II				Series III				Control	
	A		B		C		D		E	
	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.
Oct. 24	4.46		4.10		4.10		4.46		4.46	
Oct. 31	6.40		6.40		6.40		6.60		6.13	
Nov. 7	7.93		7.53		8.06		8.28		7.53	
Nov. 14	9.80		10.07		9.80		9.07		9.21	
Nov. 21	9.50	5.00	9.71	4.89	10.26	5.03	9.70	4.60	9.57	5.85
Nov. 28	10.41	6.12	9.80	6.66	10.58	7.05	9.80	6.65	10.25	8.08
Dec. 5	12.20	9.25	11.90	9.45	12.20	8.95	11.40	9.05	12.55	15.00
Dec. 12	13.20	13.05	13.45	13.45	12.70	13.25	13.20	13.30	12.88	19.05
Net total	8.74	13.05	9.35	13.45	8.60	13.25	8.74	13.30	8.42	19.05

(d) *Zea Mays*

Date	Series II				Series III				Control	
	A		B		C		D		E	
	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.
Oct. 24	Just above ground									
Oct. 31	3.50	4.62	3.62	5.18	3.38	5.19	3.50	4.62	2.88	4.77
Nov. 7	4.38	7.31	4.88	7.79	4.50	7.87	4.62	7.56	3.80	7.55
Nov. 14	5.12	7.93	5.62	9.43	5.37	9.31	5.25	8.37	5.20	8.60
Nov. 21	5.75	9.43	6.75	12.00	5.66	10.94	6.25	10.68	5.60	10.20
Nov. 28	6.75	12.93	7.85	16.35	7.11	14.61	7.37	14.31	6.40	14.10
Dec. 5	8.00	15.56	8.14	18.57	8.00	17.22	8.33	16.81	7.00	15.80
Dec. 12	9.00	18.75	9.71	20.00	9.22	20.00	9.28	18.42	8.00	17.37

(e) *Lactuca*(f) *Raphanus*

Date	Series II		Series III		Control	Series II		Series III		Control
	A	B	C	D	E	A	B	C	D	E
	Lvs.	Lvs.	Lvs.	Lvs.	Lvs.	Lvs.	Lvs.	Lvs.	Lvs.	Lvs.
Oct. 24	8.25	8.10	8.20	8.00	8.10	2.00	2.00	2.00	2.00	2.00
Oct. 31	13.35	12.35	13.15	13.15	12.65	2.80	2.90	2.30	3.30	2.90
Nov. 7	16.90	16.45	16.95	16.45	15.70	4.20	4.40	4.40	4.80	4.20
Nov. 14	20.80	20.45	21.65	20.35	20.25	5.70	6.00	5.40	5.70	5.80
Nov. 21	22.60	23.25	25.20	21.60	20.35	5.80	5.60	5.90	4.70	5.60
Nov. 28	25.61	25.27	25.05	24.52	23.11	5.80	5.60	4.90	5.10	5.37
Dec. 5	27.07	27.78	26.68	29.06	25.85	6.55	6.60	6.10	6.10	7.00
Dec. 12	28.38	27.57	32.18	30.52	26.90	7.22	7.10	6.60	6.40	7.60
Net total	20.13	19.47	23.98	22.52	18.80	5.22	5.10	4.60	4.40	5.60

(g) *Bryophyllum*

Date	Series II				Series III				Control	
	A		B		C		D		E	
	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.
Oct. 31	9.37	7.46	8.66	5.47	9.33	6.61	8.50	5.34	6.75	5.25
Nov. 7	10.37	7.62	11.00	5.81	10.11	7.05	10.00	5.87	8.00	7.18
Nov. 14	11.37	8.81	11.25	6.75	11.11	8.16	10.75	6.63	9.25	7.75
Nov. 21	12.12	9.87	11.25	7.43	11.90	9.27	11.50	8.00	10.25	7.94
Nov. 28	14.37	10.75	12.12	9.25	13.10	11.44	12.50	10.12	10.75	8.25
Dec. 5	16.28	13.85	13.5	10.68	15.22	11.50	12.75	11.25	11.25	9.25
Dec. 12	18.37	14.94	14.12	12.18	18.00	13.61	14.00	13.37	12.25	10.00
Net total	9.00	7.48	5.46	6.71	8.67	7.00	5.50	8.30	5.50	3.75

(h) *Coleus Blumei* var. *Verschaaffeltii*

Date	Series II				Series III				Control	
	A		B		C		D		E	
	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.
Oct. 24	7.66	3.75	9.16	4.08	8.83	3.83	9.50	4.00	6.83	3.25
Oct. 31	10.00	4.58	12.00	5.58	10.50	4.83	11.33	5.08	8.33	3.66
Nov. 7	13.66	5.66	17.66	7.83	14.00	6.25	17.16	6.25	11.00	4.66
Nov. 14	18.16	6.46	25.66	9.16	22.50	7.41	26.33	7.75	14.00	5.38
Nov. 21	24.50	8.33	34.00	11.25	32.40	9.40	29.33	9.50	15.50	6.16
Nov. 28	33.66	9.50	43.66	14.91	41.80	10.70	37.66	10.50	19.83	7.58
Dec. 5	40.66	10.66	51.16	16.75	53.60	12.10	43.83	12.66	25.83	10.08
Dec. 12	67.00	14.40	69.30	18.25	64.60	14.30	58.50	14.26	35.16	10.75
Net total	59.34	10.65	60.14	14.17	55.77	10.47	49.00	10.26	28.33	7.00

(i) *Coleus Blumei* var. "Spotted Gem"

Date	Series II				Series III				Control	
	A		B		C		D		E	
	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.
Oct. 24	10.10	3.33	9.66	3.50	7.00	1.66	6.00	2.00	6.00	2.00
Oct. 31	12.33	6.33	12.00	4.83	7.60	2.08	9.33	2.25	8.66	2.16
Nov. 7	19.60	6.50	20.30	6.50	9.33	2.75	11.00	3.06	10.66	3.00
Nov. 14	24.00	7.58	28.33	7.92	14.00	3.33	16.66	3.33	12.66	3.16
Nov. 21	34.00	9.33	41.00	9.83	20.00	4.50	19.00	4.75	12.66	3.83
Nov. 28	48.30	11.16	51.30	13.33	26.60	5.16	20.00	6.16	15.30	4.83
Dec. 5	58.33	13.16	64.00	16.16	32.00	5.66	22.66	6.83	18.00	6.33
Dec. 12	78.30	15.66	84.00	18.00	36.60	7.16	37.60	8.10	25.66	7.10
Net total	68.20	12.33	74.34	14.50	29.60	5.50	31.60	6.10	19.66	5.10

TABLE V

(a) SHOWING RATE OF GROWTH IN PHASEOLUS SEEDLINGS WITH BOTH COTYLEDONS PRESENT. FIGURES REPRESENT AVERAGE NUMBER OF LEAVES PER PLANT AND HEIGHT IN CENTIMETERS

Date	Series I H		Series II A		Series III C		Control E	
	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.
Oct. 28	2.00	5.25	2.00	4.37	2.00	4.66	2.00	4.21
Nov. 5	3.75	10.37	3.33	10.60	3.50	12.00	3.15	10.75
Nov. 12	5.00	12.75	4.33	13.60	5.00	13.83	4.50	13.37
Nov. 19	5.50	15.00	5.00	17.20	5.10	17.91	4.80	15.71
Nov. 26	5.50	15.80	6.00	22.80	5.30	22.91	5.00	21.08
Net total	3.50	9.55	4.00	18.43	3.30	18.25	3.00	16.87
Av. no. flowers	0		1.6		3.33		1.5	

(b) SHOWING RATE OF GROWTH IN PHASEOLUS SEEDLINGS WITH ONE COTYLEDON REMOVED

Date	Series I H		Series II A		Series III C		Control E	
	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.
Oct. 28	2.00	5.62	2.00	4.16	2.00	4.66	2.00	3.50
Nov. 5	3.25	10.37	3.00	10.40	3.10	9.16	3.30	9.20
Nov. 12	4.00	12.37	4.20	13.00	5.10	12.70	4.00	11.20
Nov. 19	5.00	14.56	4.90	16.40	5.20	13.30	4.50	13.50
Nov. 26	5.00	15.00	5.60	20.70	5.20	20.58	5.00	14.75
Net total	3.00	9.38	3.60	16.54	3.20	15.92	3.00	11.25
Av. no. flowers	0		2.4		.8		1.4	

(c) SHOWING RATE OF GROWTH IN PHASEOLUS SEEDLINGS WITH BOTH COTYLEDONS REMOVED

Date	Series I H		Series II A		Series III C		Control E	
	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.	Lvs.	Ht.
Oct. 28	2.00	5.25	2.00	3.33	2.00	5.33	2.00	4.50
Nov. 5	2.25	8.00	2.50	7.25	2.70	7.46	2.50	8.90
Nov. 12	3.30	9.87	3.60	9.08	4.80	11.00	4.20	12.62
Nov. 19	4.30	11.50	4.20	11.41	4.80	13.40	4.50	14.36
Nov. 26	4.30	11.75	5.10	15.00	4.80	18.50	4.70	18.20
Net total	2.30	6.50	3.10	11.67	2.80	13.17	2.70	13.70
Av. no. flowers	0		.5		.8		1.0	

According to Beeskow ('28) and Delf and Ritson (Delf, Ritson, and Westbrook '27) a daily exposure to ultra-violet of as long as thirty seconds produces no harmful effects in Soy beans and *Trifolium* and might cause a slight increase in growth. Thus several experiments were undertaken to see if this might not be true of other plants. Fifteen young *Nicotiana* plants were rayed at 100

inches from the light without a screen for thirty seconds each day for a period of four weeks. At the end of that time the rayed plants showed a noticeable increase in growth over the control plants (table vi). The same experiment was tried with three varieties of *Coleus* with the same results. Here the increase was not only in number of leaves, but also in height (table vi and pl. 26, fig. 2). Very young lettuce plants were also rayed with the same results.

Next, the exposure of one minute each day at 100 inches from the light was tried on the same three varieties of *Coleus*, with absolutely no change in color. There was, however, a slight retardation in the rate of growth at the end of two weeks, but even at the end of four weeks it was not very noticeable.

TABLE VI

SHOWING RATE OF GROWTH IN PLANTS RAYED 30 SECONDS EACH DAY AT 100 INCHES FROM THE LIGHT, USING NO SCREEN

Date	(a) <i>Nicotiana</i>		(b) <i>Coleus Blumei</i> var. <i>Verschaffeltii</i>			
	Rayed	Control	Rayed		Control	
	Lvs. pres.	Lvs. pres.	Lvs. pres.	Ht. in cm.	Lvs. pres.	Ht. in cm.
Jan. 24	4.26	3.93	12.25	7.50	10.60	7.83
Feb. 1	5.60	5.26	15.50	8.62	11.30	8.00
Feb. 8	7.10	7.13	23.00	10.00	16.60	9.30
Feb. 15	8.46	7.66	28.75	12.12	21.00	10.00
Feb. 22	9.93	8.66	35.45	12.75	29.00	11.30
Total	5.67	4.73	23.20	5.25	18.40	3.34

Date	(c) <i>Coleus Blumei</i> var. "Defiance"				(d) <i>Coleus Blumei</i> var. "Spotted Gem"			
	Rayed		Control		Rayed		Control	
	Lvs. pres.	Ht. in cm.	Lvs. pres.	Ht. in cm.	Lvs. pres.	Ht. in cm.	Lvs. pres.	Ht. in cm.
Jan. 24	11.00	8.37	7.00	4.00	13.50	8.62	15.75	8.87
Feb. 1	14.50	9.50	7.50	4.52	16.25	9.37	18.25	9.25
Feb. 8	19.25	11.87	8.75	6.25	32.75	10.62	33.50	9.75
Feb. 15	24.75	15.00	9.25	8.50	47.00	12.37	44.75	11.25
Feb. 22	26.50	16.50	13.75	10.37	49.75	12.75	50.50	12.00
Total	15.50	8.13	6.75	6.37	36.25	4.13	34.75	3.13

ANATOMICAL

(ALL MEASUREMENTS WERE MADE WITH AN EYEPIECE MICROMETER)

Leaves.—A cross-section of a *Cucumis* leaf rayed under the conditions present in Series I F was measured and found to be a little thinner than a corresponding section from a non-rayed leaf. When the structure of the two sections was compared, it was observed that the rayed section was lacking in upper epidermis, with only the collapsed walls remaining as a false cuticle, which no doubt gave the glossy surface to the leaf. Also the protoplasm in most of the palisade cells had drawn away slightly from the ends of the cells nearest the epidermis. The chloroplastids in the rayed section were found to be more numerous in the ends of the palisade cells nearest the spongy tissue. Another difference was the presence of fewer air-spaces in the rayed section (pl. 28, figs. 4 and 5 and table VII (b)). This corresponds well with the results found by Bailey ('94), Kluyver ('11), and Westbrook (Delf, Ritson, and Westbrook, '27).

When a cross-section of a *Cucumis* leaf, rayed as in Series II A, was measured it was found to be a little thicker than a similar section from a non-rayed plant. A leaf from Series II B was found to be still thicker. It will be remembered that this was also the group where greatest growth in height was found. A leaf from Series III C was thicker than Series II A but thinner than Series II B. A leaf from Series III D was slightly thicker than the control though not as thick as a leaf from Series II A. The control leaf showed very long palisade cells with many air-spaces between them and also among the cells of the spongy tissue. In Series II B where the thickest leaf was found, there were larger air-spaces than in the control leaf. The palisade cells in all rayed leaves in Series II and III were a little shorter than in the control leaves. The thinnest rayed leaf (Series III D) showed fewer air-spaces than the control and instead smaller and more numerous cells. The number and position of the plastids was about the same in all leaves (pl. 29, figs. 1-3, and table VII (k)).

The cross-section of a rayed leaf of *Ipomoea* from Series I H was found to be much thinner than an unrayed leaf. Here, as in *Cucumis*, the epidermis had collapsed, forming a heavy cuticle.

Occasionally, however, an epidermal cell remained intact. The protoplasm of a few of the palisade cells had drawn away from the end nearer the epidermis, and the cells themselves were shorter. A section from an *Ipomoea* leaf in Series I F proved to be of the same thickness as the one from Series I H. Here, however, the epidermis was not collapsed but thinner, each cell being absolutely distinct. The palisade cells were longer than in Series I H but not as long as the controls. There were fewer air-spaces here than in either the control leaf or the leaf in Series I H. This is probably due to the fact that Series I F was rayed for a period of eight weeks and Series I H for only four weeks (table VII (d)).

When rayed *Ipomoea* leaves from Series II A were examined, they were found to be thinner than control leaves. Leaves from Series II B were slightly thinner than those in II A. The palisade cells in both the above-mentioned groups were thinner than palisade cells in control leaves, as were also the upper epidermal cells.

Rayed leaves in Series III C about equalled the control leaves in thickness and length of palisade cells, though the upper epidermis here was still thinner than in the control leaves. Leaves from Series III D were by far the thickest in any of the series. Here the palisade cells and epidermal cells were also longer than in any of the other groups. Air-spaces were found to be much greater and more numerous than in sections of control leaves. Chloroplastids seemed to be more numerous here also (table VII (p), and pl. 29, figs. 4 and 5).

The cross-section of a rayed *Nicotiana* leaf from Series I H was again found thinner than an unrayed leaf. The same collapsing of the epidermis and shrinking of the protoplasm in the palisade cells were also found (pl. 27, fig. 7, and table VII (e)).

Rayed *Nicotiana* leaves from Series II A and B and III C were about the same thickness as those of non-rayed plants. The palisade cells from Series II A and B were a little longer than corresponding cells in a control leaf. A *Nicotiana* leaf from Series III D proved to be much thicker than one from any of the other groups mentioned. Also its palisade cells were longer, and it contained many more air-spaces. However, the upper epidermis

was thinner here than in a non-rayed leaf (pl. 27, figs. 5 and 6, and table VII (o)). It will be remembered that this group, while it did not show greatest growth in height, was the most stocky in appearance and produced flowers at the same time as did the control plants showing greatest growth in height.

When cross-sections of *Zea Mays* leaves were examined it was found that all rayed leaves in Series II and III were much thicker than non-rayed ones, the thickest being present in Series III C, which was rayed for seven weeks at 50 inches from the light using a screen of quartz-lite glass. These leaves also showed the thinnest epidermis and the best-developed vascular bundles. Leaves in Series II A and B were next in thickness, and both had well-developed bundles. Leaves in Series III D were the nearest like those of the control plants, having very thick epidermis and less well-developed bundles. In general, there seemed to be a thicker cuticle present in rayed leaves than in control leaves (pl. 32, figs. 1-6, and table VII (n)).

Cross-sections of *Coleus* leaves in Series I H which were rayed at 50 inches without a screen showed the same collapsed epidermis and lack of air-spaces as were found in similarly treated leaves of other plants. However, here in addition there was a loss of red color. Not only did the color disappear from the epidermis when it collapsed, but also from the palisade cells, showing that the rays penetrate beyond the epidermis or else produce some substance which does. This corresponds well with the results found in animal tissue by Macht, Anderson and Bell ('28) (pl. 31, figs. 4 and 5).

Non-rayed *Coleus* leaves were found to be much thicker than those rayed. The thinnest leaves were found in Series II B, where there was also the greatest growth in height. According to increasing thickness the groups may be arranged as follows; Series II B, II A, III C, III D, and last, the control E. The palisade cells were shorter in Series II B than in any of the other groups, even including the control plants. There was absolutely no decrease in red color in any of the rayed plants in Series II and III. It appeared that in *Coleus* plants the growth in height was inversely proportional to both the thickness of the leaves and the number of air-spaces present (pl. 30, figs. 1-5, and table VII (l)).

Lactuca leaves rayed as in Series I H showed the characteristic collapse of at least part of the epidermis and the lack of air-spaces. In addition, there was no differentiation of palisade tissue. Leaves rayed as in Series I F for four weeks were similar to the controls except that they were a little thinner and developed palisade and epidermal cells that were a little shorter. The air-spaces were also fewer here than in control leaves. If their leaves were rayed for eight weeks instead of four, they were still thinner, had no well-defined palisade layer, and almost no air-spaces. In thickness they about equalled the leaves in Series I H.

When rayed *Lactuca* leaves from Series II and III were examined they were all found to be of about the same thickness and much thinner than corresponding control leaves. The palisade and epidermal cells were also shorter than those present in the control leaves.

Cross-sections of rayed *Raphanus* leaves in Series I F were only very slightly thinner than those of corresponding control leaves. There was the same collapse of epidermis, forming a false cuticle as in other leaves mentioned. The contents of the upper layer of palisade cells had disappeared, leaving them empty. Leaves in Series I H showed about the same injury as those in Series I F rayed for eight weeks. Rayed leaves in Series II and III were all thinner than similar control leaves, those in Series III D being the thinnest. The palisade and epidermal cells, however, seemed to be longer than in the control leaves. This would indicate fewer air-spaces in the rayed leaves in Series II and III than in corresponding non-rayed leaves.

Sections of *Bryophyllum* leaves rayed under the conditions of Series I H were also thinner than corresponding sections of non-rayed leaves. The characteristic lack of upper epidermis was observed and also a thicker under epidermis. Sections of leaves from this plant in Series II A were much thinner than those of control leaves, and there was no collapse of upper epidermis. Leaves from Series II B were about the same thickness as control leaves, but the epidermis was slightly thinner. Leaves in Series III C were thinner than in Series II B but thicker than in Series II A. Leaves from Series III D had longer upper epidermal

cells and were much thicker than any of the other leaves including the controls.

Cross-sections of *Phaseolus* leaves in Series I F were thinner than sections from control leaves. The epidermis was destroyed in some places, but in others the epidermal cells still remained intact. The contents of the palisade cells were much shrunken and the cells shorter. The chloroplastids were also collected in the end of the cells nearest the spongy tissue. Sections of *Phaseolus* leaves from Series II A were much thicker than control sections. They also had longer palisade and epidermal cells and more numerous air-spaces and chloroplastids. *Phaseolus* leaves from Series III C were also thicker than non-rayed leaves but not as thick as those in Series II A (pl. 28, figs. 1-3, 6, and table VII (c and m)).

TABLE VII

SHOWING THICKNESS OF LEAVES AND LENGTH OF CELLS IN MILLIMETERS FOR RAYED AND CONTROL PLANTS. CELL LENGTH TAKEN NORMAL TO LEAF SURFACE

	(a) <i>Lactuca</i>				(b) <i>Cucumis</i>		(c) <i>Phaseolus</i>		(d) <i>Ipomoea</i>		
	Con.	Series I			Con.	Series I	Con.	Series I	Con.	Series I	
	G	F	F'	H	G	F	G	F	G	F'	H
Leaf	.1279	.101	.076	.074	.1247	.1226	.0938	.0814	.1565	.117	.117
Palisade	.0298	.021	.0156	.014	.045	.045	.0384	.0296	.0408	.034	.029
Upper epid.	.0138	.0137	.0109	.0107	.0135	(—)	.0119	(—) or .0114	.0243	.012	(—)
Lower epid.	.0107	.0091	.0107	.009 or (—)†	.0068	.0126	.0098	.0102	.0239	.011	.0145

	(e) <i>Nicotiana</i>		(f) <i>Bryophyllum</i>		(g) <i>Raphanus</i>		(h) <i>Raphanus</i>				
	Con.	Series I	Con.	Series I	Con.	Series I	Series II		Series III		Con.
	G	H	G	H	G	F'	A	B	C	D	E
Leaf	.143	.1326	.460	.4338	.1604	.1612	.1582	.158	.149	.1293	.163
Palisade	.0525	.0529	(—)	(—)	.0495	.0635	.0495	.049	.047	.0411	.038
Upper epid.	.0218	(—)	.0153	(—)	.0142	(—)	.0148	.019	.015	.0134	.013
Lower epid.	.011	.0112	.0121	.0145	.0151	.0123	.01176	.0103	.009	.0117	.007

	(i) <i>Lactuca</i>					(j) <i>Bryophyllum</i>				
	Series II		Series III		Control	Series II		Series III		Control
	A	B	C	D	E	A	B	C	D	E
Leaf	.0884	.087	.0896	.0882	.1013	.360	.522	.4464	.8075	.5103
Palisade	.0182	.0207	.0204	.0176	.0263					
Upper epid.	.0128	.0154	.012	.0134	.0154	.0153	.0189	.0202	.0153	.0207
Lower epid.	.0078	.0109	.0092	.0089	.0126	.0121	.0099	.0126	.0144	.0121

* F, plants rayed 8 weeks at 100 inches without a screen.

† (—), lacking.

	(k) <i>Cucumis</i>					(l) <i>Coleus</i>				
	Series II		Series III		Control	Series II		Series III		Control
	A	B	C	D	E	A	B	C	D	E
Leaf	.1307	.1397	.1363	.127	.1257	.1181	.1056	.1232	.1374	.138
Palisade	.0448	.0459	.0454	.0453	.0484	.0364	.0299	.037	.0371	.0375
Upper epid.	.0134	.0096	.0117	.0133	.0126	.021	.0184	.0159	.0156	.0168
Lower epid.	.007	.0117	.007	.0071	.0072	.0086	.0078	.0103	.007	.0112

	(m) <i>Phaseolus</i>					(n) <i>Zea Mays</i>				
	Series II		Series III		Control	Series II		Series III		Control
	A	B	C	D	E	A	B	C	D	E
Leaf	.1369		.1016		.0915	.1408	.1402	.1467	.1366	.1195
Palisade	.0518		.038		.036					
Upper epid.	.018		.0111		.010	.0263	.0260	.020	.0296	.0274
Lower epid.	.012		.0069		.0067	.0204	.0304	.026	.0196	.019

	(o) <i>Nicotiana</i>					(p) <i>Ipomoea</i>				
	Series II		Series III		Control	Series II		Series III		Control
	A	B	C	D	E	A	B	C	D	E
Leaf	.143	.143	.1444	.1584	.145	.1206	.1184	.1316	.168	.133
Palisade	.0525	.0498	.0362	.0487	.0414	.0355	.0375	.0467	.0604	.0476
Upper epid.	.0218	.019	.0148	.0162	.0207	.0179	.0193	.0188	.0243	.0226
Lower epid.	.011	.0112	.0142	.0086	.0103	.0168	.017	.0176	.0212	.017

Stems.—Cross-sections from the base of non-rayed *Cucumis* stems seven weeks old were found to have smaller diameters than any of those from rayed stems. Similar sections from the base of *Cucumis* stems in Series II A and III D were found to be a little broader, while those in Series II B and III C had the

greatest diameter (table VIII). Series II B had the thickest leaves and the greatest growth in height. All rayed *Cucumis* stems in Series II and III also showed larger bundles than control stems, though there was little difference between the different rayed groups. The average width of the rayed bundles from the outside of the stem toward the center was 0.54 mm. and that of similar control bundles was 0.36 mm. The amount of bast tissue was about the same for control as for rayed stems.

Cross-sections from the base of stems of control *Nicotiana* plants were also found to be smaller in diameter than most of the rayed ones. Series II A developed stems a little smaller in diameter than those of the control plants. Stems in Series II B and III C were larger in diameter, and those in Series III D were still larger. It will be remembered that this was the group of tobacco plants that showed the thickest leaves and the healthiest appearance (table VIII). As to development of vascular tissue the control plants again had the thinnest vascular cylinders which were 0.36 mm. in thickness. Next in order of thickness came Series II A (0.378 mm.) followed by Series III C (0.505 mm.) and III D (0.54 mm.). The tracheae were also smallest in the control plants and largest in Series III D with Series II A, B, and III C intermediate and equal. In all cases the walls of the tracheae were thicker in rayed plants than in non-rayed ones.

When sections from the bases of rayed *Zea Mays* stems of the different series were measured, it was found that those in Series II A were a little smaller in diameter than those from the control stems. Series II B had stems a little larger and III D still larger than those in Series II B. Stems in Series III C were the largest of all. This was also the group of plants that showed the thickest leaves and the greatest growth.

There was a noticeable range of variation present in the vascular bundles of the different groups of *Zea Mays* plants. Rayed stems in Series II A showed bundles smaller than those of the control stems, both as to entire bundle and as to size of vessels, but the phloem was better developed than in control stems. Series II B showed bundles of about the same size as control stems, but here the phloem was as well developed as in Series II A and the mechanical tissue much better developed than in

either the control stems or those in Series II A. The best-developed bundles were found in Series III C. Here the phloem and mechanical tissue were very well developed. The walls of the vessels were much heavier here than in any other group of *Zea Mays* plants. The pith cells in the control plants and those in Series II A were angled, while those in Series II B and III C and D were oval in shape, showing more air-spaces. The oval pith cells were also larger than corresponding angled ones. Series III C was also found to have many more layers of cells making up the cortex than any of the other groups of *Zea Mays* plants (pl. 33, figs. 1-8, and table VIII).

When sections of *Coleus* stem from the different groups of plants were measured, it was found that the control plants again showed smaller stems than any of the rayed plants. The stems largest in diameter were found in Series II A and B. It was these two groups also that showed the greatest growth in height (table VIII). The radial diameter of the vascular bundles of the control stems was 0.495 mm., while that of Series III D was 0.612 mm., that of III C, 0.62 mm., and of Series II B, 0.675 mm. This corresponds well with the fact that greatest growth in height was found in this group. Bast tissue was present about equally in all rayed and control stems of *Coleus*.

Sections of *Phaseolus* also showed the control plants to have stems smaller in diameter than any of the rayed plants. Series II A has stems having the greatest diameter. It will be remembered that this group of *Phaseolus* plants also showed the thickest leaves. Series III C had stems just a little smaller in diameter than those in Series II A (table VIII). When the

TABLE VIII
SHOWING THICKNESS IN MILLIMETERS OF RAYED AND NON-RAYED
STEMS

Plant	Series II		Series III		Control
	A	B	C	D	E
Cucumis	5.0	5.5	5.5	5.0	4.0
Nicotiana	8.8	9.5	9.5	10.0	8.5
Zea Mays	8.0	8.8	10.7	9.5	8.5
Coleus	6.0	6.0	5.5	5.5	4.8
Phaseolus	3.8		3.5		3.0

vascular cylinder in the different rayed groups was compared it was found to be by far the thickest in Series II A and III C, averaging 0.54 mm. in diameter, while that of the control stems averaged 0.49 mm.

PHYSIOLOGICAL

Chlorophyll decomposition.—A medium green 80 per cent alcoholic chlorophyll solution was made from *Nicotiana* leaves and put in test-tubes of pure fused silica. These were placed horizontally in white dishes and exposed to the different conditions, with results given in table ix.

TABLE IX
SHOWING THE AMOUNT OF TIME NEEDED TO DECOLORIZE CHLOROPHYLL SOLUTION

	9 a.m.	12 m.
Sunlight in greenhouse.....	12 min.	6 min.
Sunlight outside.....	4 min.	2 min.
Diffused light in greenhouse.....	35 min.	18 min.
At 30 inches from an unscreened lamp in diffused light.....	39 min.	20 min.
Ultra-violet lamp screened with vita glass plus diffused light.....	40 min.	22 min.
Sunlight outside under a screen of vita glass.....	6 min.	3 min.
Sunlight outside under a screen of quartz-lite glass.....	5 min.	2½ min.

These results show plainly that ultra-violet rays do not hasten the decomposition of chlorophyll. Vita glass is thicker than quartz-lite, and hence used at close range the difference in thickness would account for the longer time required for the decomposition under vita glass in sunlight.

Starch storage.—Sections of *Coleus* stem at the end of seven weeks showed more starch in control plants than in any of the plants rayed as in Series II and III. It was impossible, however, to distinguish between the different rayed stems.

In sections of *Phaseolus* stem starch was present in the cortex of plants rayed as in Series II A, while similar control plants showed very little if any starch in the cortex.

Determination of P_H .—*Lactuca* plants under experiment for eight weeks as in Series I F were used for this work, the leaves and stems being determined separately. The P_H of the leaves and stems of both rayed and control plants was found to be 6.0.

The P_H of leaves and roots of *Raphanus* was determined sepa-

ately, and here again the rayed and control plants responded alike, that of the leaves being 6.2 and the roots 6.0. Thus raying with ultra-violet rays seems to have no effect on the P_H of plants.

Dry weights.—In the experiments carried on in 1926 to 1927 as described in Series I, which consisted of plants rayed with an unscreened lamp, dry weight determinations were made of the entire tops of *Lactuca* and both tops and roots of *Raphanus*. In all cases greater dry weight was found in the control plants. This can be well seen in the results in table x (a) and (b).

TABLE X
SHOWING IN GRAMS THE DRY WEIGHT OF PLANTS IN SERIES I H
(50 INCHES), F (100 INCHES), AND G (CONTROL)

(a) <i>Raphanus</i>						
	4 weeks		4 weeks		8 weeks	
	H	G	F	G	F	G
Tops	.36	.69	.326	.49	.586	1.0
Roots	.41	.79	.126	.174	.24	.496

(b) Tops of <i>Lactuca</i> plants								
	2 leaves		9 leaves		2 leaves		9 leaves	
	H	G	H	G	F	G	F	G
4 weeks	.018	.244	.805	2.22	.116	.24	.81	1.24
8 weeks	.003	.770	.825	3.85	.200	1.15	1.56	2.76

In the experiments carried on in 1927 to 1928 the dry weight was determined for fifty grams of wet weight of leaves.

Rayed *Zea Mays* plants of Series II and III showed greater dry weight than corresponding control plants. Series II A showed the smallest dry weight of the rayed plants which was where the poorest growth in rayed plants of Series II and III was found.

Lactuca plants in Series III D had the greatest dry weight. Those in Series II A and III C showed smaller dry weight than the control plants.

Ipomoea plants in Series III D had the greatest dry weight. It was also in this group that greatest growth and thickest leaves were found.

Nicotiana plants showed greatest dry weight in the control plants and the smallest in Series II A.

Cucumis plants had the smallest dry weight in Series II B, and in this group were the greatest growth and thickest leaves with the largest air-spaces.

Phaseolus plants in Series II A showed the greatest dry weight and also the thickest leaves.

Plants of *Raphanus* showed the greatest weight in Series III C and D and the next greatest in the control plants.

Bryophyllum plants also had the greatest dry weight in Series III C and D, though all rayed plants in Series II and III had greater weights than similar control plants. A comparison of the dry weights of the various plants will be found in table XI.

TABLE XI

SHOWING THE DRY WEIGHT IN GRAMS PER FIFTY GRAMS OF WET WEIGHT OF PLANTS IN SERIES I (RAYED WITHOUT A SCREEN), SERIES II (SCREEN OF VITA GLASS) AND SERIES III (SCREEN OF QUARTZ-LITE GLASS)

Plant	Series I	Series II		Series III		Control
	H	A	B	C	D	E
Zea Mays	6.0331	5.462	6.1496	5.8290	5.9232	5.2030
Lactuca	3.1950	2.2307	2.8396	2.3322	3.2320	2.7959
Ipomoea		7.4775	7.0642	7.6735	7.7290	7.3554
Nicotiana		5.3150	5.5130	5.9240	5.9675	6.3225
Cucumis		4.8072	4.5494	4.7149	4.7544	4.8420
Phaseolus		6.9050		5.3994		6.3695
Raphanus		3.8845	3.9775	4.3200	4.3410	4.1361
Bryophyllum	2.8295	3.5100	3.4055	4.2975	3.7585	3.5286

Ash determination.—The results were not conclusive, but they point toward an increase in ash in plants rayed with an unscreened lamp. In plants rayed with a screened lamp the ash was, in the majority of cases, less than in the control plants. In *Cucumis* plants showing best growth there was less ash and also smaller dry weight than in the control plants. This can be explained, however, by the presence of many large air-spaces in those leaves while in the control leaves the air-spaces were smaller.

In *Phaseolus* the amount of ash again corresponded very well with the dry weights, there being the greatest dry weight where there was the greatest amount of ash. This also corresponded with the thickness of the leaves. For comparison of the results see table XII.

TABLE XII

SHOWING THE WEIGHT IN GRAMS OF ASH FOR 3 GRAMS OF DRY LEAF MATERIAL

Plant	A	B	C	D	E	F
Zea Mays	.310	.272	.269	.295	.325	.349
Lactuca	.600	.610	.611	.581	.595	.565
Ipomoea	.410	.302	.395	.392	.505	
Nicotiana	.527	.533	.505	.515	.570	
Cucumis	.562	.521	.505	.530	.599	
Phaseolus	.464		.435		.455	
Raphanus	.638	.630	.643	.558	.789	
Bryophyllum	.564	.5193	.482	.430	.540	.610

The effect of ultra-violet radiation upon transpiration.—When leaves of *Phaseolus*, *Cucumis*, *Lactuca* and *Coleus* were placed in bottles of water, sealed with paraffin, and rayed at 50 inches from the unscreened lamp, it was found through weighings taken every 30 minutes of bottles and leaves combined that at first the rayed leaves lost as much as the controls. Then there was a time when less weight and sometimes no weight was lost by rayed leaves. After that there was a loss equalling that of the control leaves kept in darkness or in some cases surpassing it. When the stomata were examined at the end of three hours those in the rayed leaves were found to be closed, while those in the leaves kept in darkness were partly open. When the rayed and control leaves were weighed at the beginning and end of the experiment, it was found that all the rayed leaves had lost weight while the controls had remained constant (fig. 2). It will be noted that *Coleus* behaved a little differently than did the other leaves used. This might be explained by the fact that *Coleus* has stomata on the under surface only. This experiment was repeated several times with the different leaves.

The petioles of leaves of *Coleus Blumei* var. *Verschaffeltii* were paraffined and the leaves placed in a horizontal position, some being rayed on the upper side, some on both sides, and others placed in darkness. Those in darkness and those rayed on the upper surface were partly wilted at the end of twelve hours while those rayed on both sides were still turgid. When weighed, however, the leaves rayed on both sides and those upon one side only were found to have lost much more weight than the leaves kept in darkness (table XIII, and pl. 26, fig. 3).

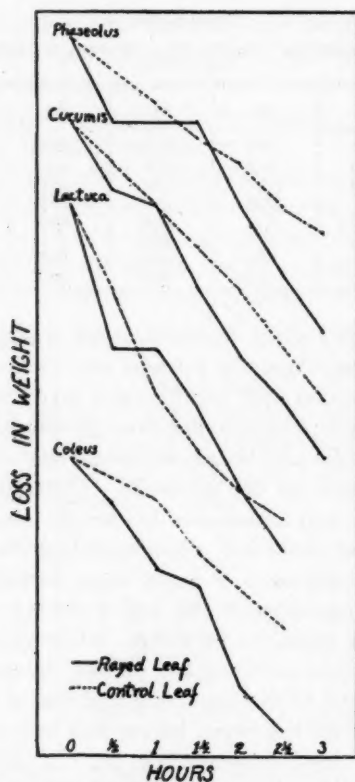


Fig. 2. Showing the comparison between the loss of weight of leaves rayed with an unscreened lamp and those kept in darkness.

TABLE XIII
SHOWING LOSS OF WEIGHT IN GRAMS OF COLEUS LEAVES WITH
PARAFFINED STEMS

	Control	Rayed on both sides	Rayed on one side
Original weight	1.90	2.10	1.65
Weight after 12 hours	1.75	1.72	1.20
Loss	.15	.38	.45

DISCUSSION

Several points have been very clearly brought out by the foregoing experiments. All plants rayed with an unscreened

quartz mercury lamp were conspicuously injured. At a distance of 50 inches from such a lamp the injury was very great to all plants. At 100 inches away the injury was not so great for the same length of exposure, probably due to a portion of the injurious rays being absorbed by the atmosphere between the lamp and the plant. It was also evident that at this distance some plants were more resistant to ultra-violet radiation as a whole than others and that younger plants were less resistant than older ones. The latter fact was particularly noticeable in *Lactuca* where the growth of young plants was almost completely stopped while that of older ones was only retarded.

The injury was first evident in the epidermis where many of the cells if not collapsed, forming a false cuticle, were smaller than those of control leaves. After raying for a period of weeks, the injury to newly formed leaves was evident through the entire leaf, causing the mesophyll tissue to be more compact with fewer air-spaces and with little differentiation between the different kinds of cells. In plants such as *Raphanus* the contents of the palisade cells were drawn away from the upper ends of the cells, particularly in regions where the epidermis had collapsed. These results suggest that raying with an unscreened lamp may actually retard growth in individual cells even if it does not kill them.

Bailey ('94), using an open arc lamp, and Kluyver ('11), Ritson and Westbrook (Delf, Ritson and Westbrook, '27), using quartz mercury vapor lamps, obtained similar results though different methods of raying were used in all cases. Bailey and Kluyver found anthocyanin disappearing from rayed *Coleus* leaves when the color was present in the epidermis only.

In the foregoing experiments when *Coleus* was rayed under the same general conditions, the anthocyanin pigment disappeared also from the palisade cells of the leaves and from the entire stem tips.

Until recently the penetration of the short ultra-violet rays was thought to be very slight. In fact a layer of skin was said to inhibit their passage. Macht, Anderson, and Bell ('28), however, using living anesthetized animals have shown that rays as short as $313\text{ }\mu$ penetrate into the peritoneal cavity of a rabbit.

Therefore it might not be unusual for rays to penetrate through plant epidermis into the palisade cells or into the cortex of a stem tip, either destroying the pigment or preventing its formation. Green ('97) has suggested that chlorophyll might act as a screen absorbing injurious rays. This supposition is strengthened by the results of experiments on chlorophyll in this paper where ultra-violet rays were found to have very little, if any, effect upon chlorophyll decomposition. If there is a screening action of chlorophyll, it might not have been evident here due to the small number of chloroplastids present in the cortex of the stem. On the other hand, the rays might not have penetrated deep enough to cause direct action, but may have set up chemical reaction which induced the decomposition of anthocyanin or prevented its formation.

Sheard and Higgins ('27) found that in general rays of 270 to 320 μ delayed the time of germination and lessened the rate of growth, but that rays of 320-390 μ were effective in promoting growth.

In the experiments in this paper where the lamp was screened by vita glass which cut out rays below 290 μ , there were no lesions though the newly formed leaves of *Lactuca*, *Raphanus*, and *Coleus* were thinner. In the other plants used the leaves were either of the same or greater thickness than control leaves.

When the lamp was screened by quartz-lite glass which cut out rays shorter than 310 μ there was also no evidence of lesions though the same three plants again had thinner leaves, while all others had much thicker leaves than the control plants.

In nearly all cases where leaves were found to be thicker when rayed by a screened lamp, the plants themselves were found to be taller with larger stems and more numerous leaves. In no cases was flower production retarded, and in *Cucumis* and *Phaseolus* it was slightly increased.

Sometimes the increase in size took the form of more numerous and larger air-spaces with only slightly larger cells. This was true of both the stems and leaves of *Cucumis* and *Nicotiana* and of the stems of *Zea Mays*. In other cases there were more air-spaces but when this occurred the palisade cells were very much larger. This was very noticeable in *Ipomoea* and *Phaseolus*. In

the leaves of *Zea Mays* the increased growth was evident only in the form of larger cells.

Coleus plants rayed with a lamp screened by vita glass or quartz-lite glass showed a marked increase in growth over control plants, though the thickness of the leaves was inversely proportional to their increase in height and number. This might indicate incipient injury to the leaves with a possible stimulatory effect upon the plant as a whole. In addition there was no loss of red color here as there had been when an unscreened lamp was used.

The results with the use of screens correspond well with those of McCrea ('27), where increased growth was obtained in *Digitalis* by the use of vita glass instead of ordinary glass in a greenhouse, and with those of Tsuji ('18), who produced larger and juicier pineapples by raying them in the field with a weak ultra-violet lamp.

When *Phaseolus* plants with both cotyledons removed were rayed either by a screened or an unscreened lamp there was a retardation in growth compared with corresponding non-rayed plants. When only one cotyledon was removed from *Phaseolus* plants, raying with a screened lamp produced a slight increase in growth over corresponding non-rayed plants. This compares well with the work of Westbrook (Delf, Ritson, and Westbrook, '27) where different lengths of day were used in addition to a short daily raying with an unscreened lamp. In all cases the injury was greater the shorter the day. These results and the fact that older *Lactuca* plants were more resistant than young ones may indicate the possibility that the presence of sufficient food may at least partially overcome the injurious effects of raying.

Clement ('26) found that apples that had been rayed stayed turgid longer than non-rayed ones. Likewise Tsuji ('18) observed that stalks of banana plants that were rayed after being cut kept fresh many days longer than non-rayed ones. The writer has found that if the petioles of *Coleus* leaves were dipped in paraffin and the leaves then rayed with an unscreened lamp first on one side and then on the other for a period of one and a half hours each, they remained turgid at the end of twelve hours, while the control leaves were badly wilted. If, however, the leaves were rayed only on one side the leaves were much more

wilted than the control leaves. All these observations would tend to indicate that a false cuticle is formed wherever plant tissue is rayed heavily with ultra-violet, thus greatly retarding the rate of water loss from the internal tissues.

In the foregoing experiments, using a wide variety of plants in sufficient numbers to avoid individual differences, and using two well-known ultra-violet glasses as screens, the writer has reached the conclusion that increased growth can be obtained in many groups of plants by the daily use of a quartz mercury vapor lamp screened to cut out the harmful short rays. However, results point to the supposition that each variety of plant has its own ultra-violet requirement for best growth and that this can be determined only by experiment.

SUMMARY

1. Raying with an unscreened quartz mercury vapor lamp caused injury in all plants used.
2. Raying with a lamp screened by vita glass was beneficial for some plants, while it produced little visible effect in others. When examined anatomically no lesions were present, but in some cases there was a slight retardation of growth.
3. Raying with a lamp screened by quartz-lite glass injured none and benefited many of the plants. In some cases, however, the benefit was less than when vita glass was used.
4. Except for *Raphanus* and possibly *Lactuca* the healthiest-appearing plants were among those rayed with a screened lamp, although the distance from the light and the screen promoting best growth differed for different plants.
5. Raying with a screened lamp increased flower production slightly.
6. Plants rayed for a period of weeks with an unscreened lamp developed leaves which were thinner than those of corresponding non-rayed plants, the decrease in thickness being due to a partial or complete collapse of the upper epidermal cells, a lack of differentiation of the palisade layer, and a decrease in the number and size of the air-spaces present in the mesophyll tissue.
7. With the exception of *Coleus*, *Raphanus*, and *Lactuca*, leaves of plants rayed with a screened lamp were in general thicker than

corresponding leaves from control plants, though the particular screen and distance from the lamp promoting the formation of the thickest leaves differed for different plants. The increase in thickness was due either to increase in size of cells or to increase in number and size of air-spaces or to both.

8. The stems rayed with a screened lamp were greater in diameter and contained better-developed vascular bundles than non-rayed ones.

9. A limitation of the amount of available food emphasizes the injurious effect of ultra-violet rays.

10. Ultra-violet radiation had very little, if any, effect upon the decomposition of chlorophyll and thus very little effect upon the photosynthetic apparatus.

11. Ultra-violet radiation had no effect upon the P_H of the plants used.

These results again emphasize the fact that each plant has its own ultra-violet requirement for best growth which can be determined only by experiment.

ACKNOWLEDGMENTS

The writer wishes to express sincere appreciation to Dr. B. M. Duggar, Professor of Physiological and Applied Botany, University of Wisconsin, formerly Professor of Plant Physiology in the Henry Shaw School of Botany of Washington University, for suggesting this problem and under whose guidance the early part of this work was carried out; to Dr. E. S. Reynolds, Physiologist to the Missouri Botanical Garden, for his advice and helpful criticisms concerning the work; to Dr. C. F. Hagenow, of the Physics Department of Washington University, for spectral photographs; to Dr. LeRoy McMaster, of the Chemistry Department of Washington University, for the use of laboratory and apparatus; and to Dr. G. T. Moore, Director of the Missouri Botanical Garden, for the privileges and facilities of that institution.

BIBLIOGRAPHY

- Bailey, L. H. ('94). Electricity and plant growing. *Mass. Hort. Soc. Trans.* 1894: 1-28. 1894.
Barr, C. E. and W. T. Bovie ('23). Ultra-violet cytology of protoplasm. *Jour. Morph.* 38: 295-300. 1923.

- Bassoni, C. B. ('14). The destruction of bacteria through the action of light. *Am. Jour. Pub. Health* 4: 915-992. 1914.
- Beeskow, H. C. ('27). Some physiological reactions of ultra-violet rays on plants. Report given at Nashville meeting of Am. Soc. Plant Physiol. Dec. 1927.
- Bovie, W. T., and G. A. Daland ('23). New experiments on the sensitization of protoplasm to heat by exposure to light of short wave-length. *Am. Jour. Physiol.* 66: 55-66. 1923.
- Brooks, Matilda ('26). Effect of light of different wave lengths on penetration of 2,6,- dibromo phenol indophenol into Valonia. *Soc. Exp. Biol. & Med. Proc.* 23: 576-577. 1926.
- Burge, W. E. ('17). Action of ultra-violet radiation in killing living cells such as bacteria. *Am. Jour. Physiol.* 43: 429-432. 1917.
- Calabek, J. ('27). Ultra-violet rays and the swelling of agar-agar. *Protoplasma* 3: 17-40. 1927.
- Clement, H. ('26). Curieux effets des rayons fournis par une lampe à vapeurs de mercure sur des pommes. *Soc. Biol. Compt. Rend.* 94: 862. 1926.
- Dane, H. Rebecca ('27). The effect of ultra-violet radiation upon soybeans. *Science N. S.* 66: 80. 1927.
- Delf, E. M., K. Ritson, and A. Westbrook ('27). The effect on plants of radiations from a quartz mercury vapor lamp. *Brit. Jour. Exp. Biol.* 5: 138-154. 1927.
- Downs and Blunt ('77). *Roy. Soc. London, Proc.* 26: 488. 1877. [cited by Ellis and Wells, '25.]
- Ellis, C., and A. A. Wells ('25). The chemical action of ultra-violet rays. pp. 1-362. New York, 1925.
- Green, R. ('97). On the action of light on diastase and its biological significance. *Roy. Soc. London, Phil. Trans.* 188 B: 167-190. 1897.
- Henri, V. ('12). Comparaison de l'action des rayons ultra-violet sur les organismes avec les réactions photochimiques simples et complexes. *Soc. Biol. Compt. Rend.* 73: 323-325. 1912.
- Hess, A. ('26). The newer knowledge of the physiological action of ultra-violet rays. *Am. Phil. Soc. Proc.* 65: 202-206. 1926.
- Hill, L. ('27). Measurement of the biologically active ultra-violet rays of sunlight. *Roy. Soc. London, Proc. B* 102: 119-128. 1927.
- Kluyver, A. J. ('11). Beobachtungen über die Einwirkung von ultravioletten Strahlen auf höhere Pflanzen. *K. Akad. Wiss. Wien, Sitzungsber.* 120: 1137-1170. 1911.
- Luckiesch, M. ('27). Ultra-violet radiation. pp. 1-258. New York, 1927.
- Macht, D. I., W. T. Anderson, and F. K. Bell ('28). The penetration of ultra-violet rays into live animal tissue. *Am. Med. Assoc. Jour.* 90: 161-165. 1928.
- Mashimo, T. ('19). A method of investigating the action of ultra-violet rays on bacteria. *Kyoto Imp. Univ. Coll. Sci. Mem.* 4: 1-11. 1919.
- McCrea, Adelia ('27). The effect of ultra-violet light on *Digitalis purpurea*. Report given at Nashville meeting of Bot. Soc. Am., Dec. 1927. Also *Science N. S.* 67: 277-278. 1928.
- Nadeon, G., et E. Rochline-Gleichgewicht ('28). Apparition des cristaux d'oxalate de calcium dans les cellules végétales sous l'influence de la radiation ultra-violette. *Soc. Biol. Compt. Rend.* 98: 363-365. 1928.
- , et G. Philippov ('28). Action excitante des rayons ultra-violet sur le développement des levures et des moisissures. *Ibid.* 366-368. 1928.

- Popp, W. H. ('26). A physiological study of the effect of light of various ranges of wave length on the growth of plants. *Am. Jour. Bot.* 13: 706-735. 1926.
- , ('26). Effect of light intensity on growth of soybeans and its relation to the autocatalyst theory of growth. *Bot. Gaz.* 82: 306-319. 1926.
- Potthoff, P. ('20). Über die Einwirkung ultravioletter Strahlen auf Bakterien und Bakteriensporen. *Doct. Diss. Univ. Gottingen.* 1920.
- Russell, E. H. and W. K. Russell ('27). Ultra-violet radiation and actinotherapy. pp. 170-202. New York, 1927.
- Schanz, F. ('20). Concerning the effect of ultra-violet rays of day light on vegetation. *Pflüger's Archiv.* 181: 229-248. 1920.
- Sheard, C., and G. M. Higgins ('27). The influence of selective and general radiations by a quartz mercury lamp upon germination and growth of seeds. *Science N. S.* 65: 282-284. 1927.
- Stoklasa, A. ('11). Über den Einfluss der ultra-violetten Strahlen auf die Vegetation. *Centralbl. f. Bakt. II Abt.* 31: 477. 1911.
- , ('15). Über die Bedeutung der Einwirkung der ultravioletten Strahlen auf die photochemische Synthese der Kohlenhydrate in der Chlorophyll haltigen Zelle. *Zentralbl. f. Biochem. und Biophys.* 18: 370. 1915.
- Tanner, F. W., and E. Ryder ('23). Action of ultra-violet light on yeast-like fungi. *Bot. Gaz.* 75: 309-317. 1923.
- Tshuhotine, S. ('23). Sur la mecanisme de l'action des rayons ultra violets sur la cellule. *Inst. Pasteur, Ann.* 35: 321-325. 1923.
- Tsuji ('18). Stimulation in growth of sugar cane and increase of percentage of sugar on exposure to ultra-violet rays. *La. Planter* 60: 413. 1918.
- Ursprung, A., und G. Blum ('17). Über die Schädigkeit ultravioletten Strahlen. *Ber. d. deut. Bot. Ges.* 35: 385-402. 1917.

EXPLANATION OF PLATE

PLATE 21 (SERIES I F, H AND G)

Fig. 1. *Cucumis sativus*.

- A. Plant rayed four weeks at 100 inches from the light without a screen.
- B. Plant not rayed.

Fig. 2. *Cucumis sativus*.

- A. Plant not rayed.
- B. Plant rayed for eight weeks as in fig. 1 A.

Fig. 3. *Raphanus sativus*.

- A. Plants not rayed.
- B. Plants rayed for eight weeks at 100 inches from the light without a screen.
- C. Plants rayed for eight weeks at 50 inches from the light without a screen.

Fig. 4. *Raphanus sativus*.

- A. Plant rayed for eight weeks at 100 inches as in fig. 3 B.
- B. Plant not rayed.

Fig. 5. *Raphanus sativus*.

- A. Plant rayed for four weeks at 100 inches as in fig. 3 B.
- B. Plant not rayed.

Fig. 6. *Ipomoea Batatas*.

- A. Plant rayed for eight weeks at 100 inches from the light without a screen.
- B. Plant not rayed.

Fig. 7. *Raphanus sativus*.

- A. Plant not rayed.
- B. Plant rayed for eight weeks as in fig. 3 B.



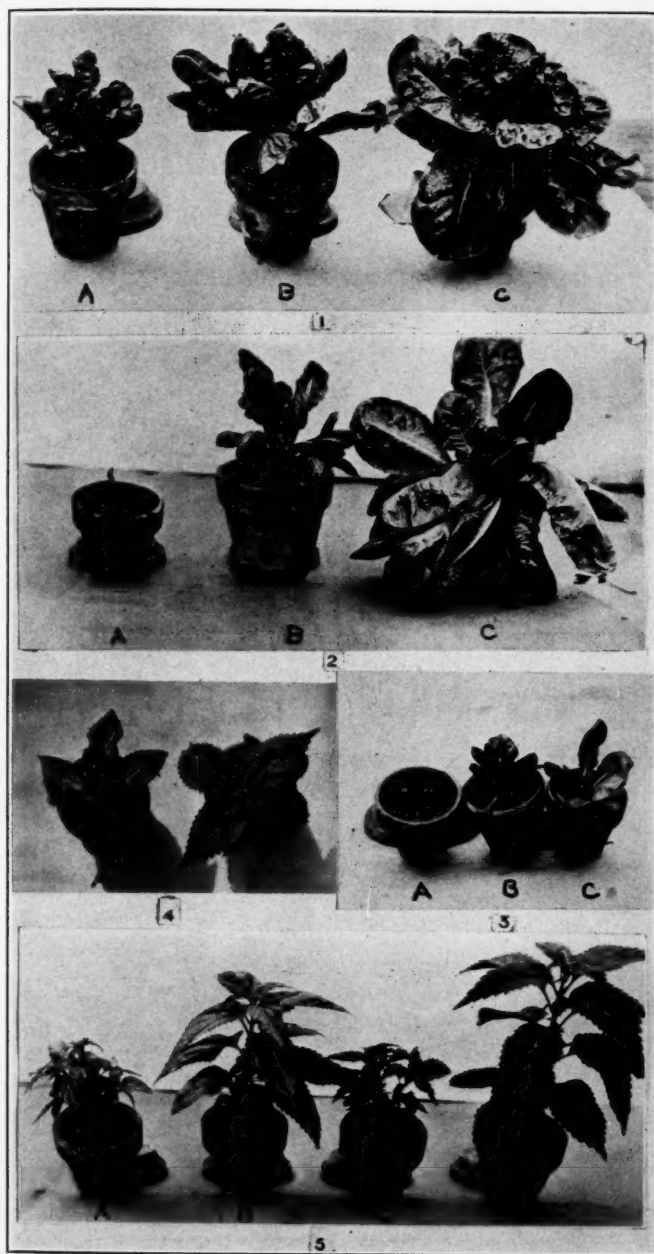
ELTINGE—EFFECT OF ULTRA-VIOLET RADIATION



EXPLANATION OF PLATE

PLATE 22 (SERIES I H, F AND G)

- Fig. 1. *Lactuca sativa* (nine leaves) rayed for four weeks.
A. Plant rayed at 50 inches from the light without a screen.
B. Plant rayed at 100 inches from the light without a screen.
C. Plant not rayed.
- Fig. 2. *Lactuca sativa* (two leaves) rayed for eight weeks.
A. Plant rayed at 50 inches from the light without a screen.
B. Plant rayed at 100 inches from the light without a screen.
C. Plant not rayed.
- Fig. 3. *Lactuca sativa* (two leaves) rayed for four weeks.
A. Plant rayed at 50 inches from the light.
B. Plant rayed at 100 inches from the light without a screen.
C. Plant not rayed.
- Fig. 4. *Coleus Blumei* var. "Spotted Gem."
A. Plant rayed for two weeks at 50 inches from the light without a screen.
B. Plant not rayed.
- Fig. 5. *Coleus Blumei* vars. *Verschaffeltii* and "Spotted Gem."
A. Var. "Spotted Gem" rayed for eight weeks at 100 inches from the light without a screen and then allowed to recover in the greenhouse for four weeks.
B. Var. "Spotted Gem" not rayed.
C. Var. *Verschaffeltii* treated the same as in fig. 5 A.
D. Var. *Verschaffeltii* not rayed.



ELTINGE—EFFECT OF ULTRA-VIOLET RADIATION



EXPLANATION OF PLATE

PLATE 23 (SERIES I F AND G)

Fig. 1. *Coleus Blumei* vars. "Spotted Gem" and *Verschaaffeltii*.

- A. Var. "Spotted Gem" rayed for four weeks at 100 inches from the light without a screen.
- B. Var. "Spotted Gem" not rayed.
- C. Var. *Verschaaffeltii* rayed under the same conditions as "Spotted Gem."
- D. Var. *Verschaaffeltii* not rayed.

Fig. 2. *Coleus Blumei* var. "Spotted Gem" and *Verschaaffeltii*.

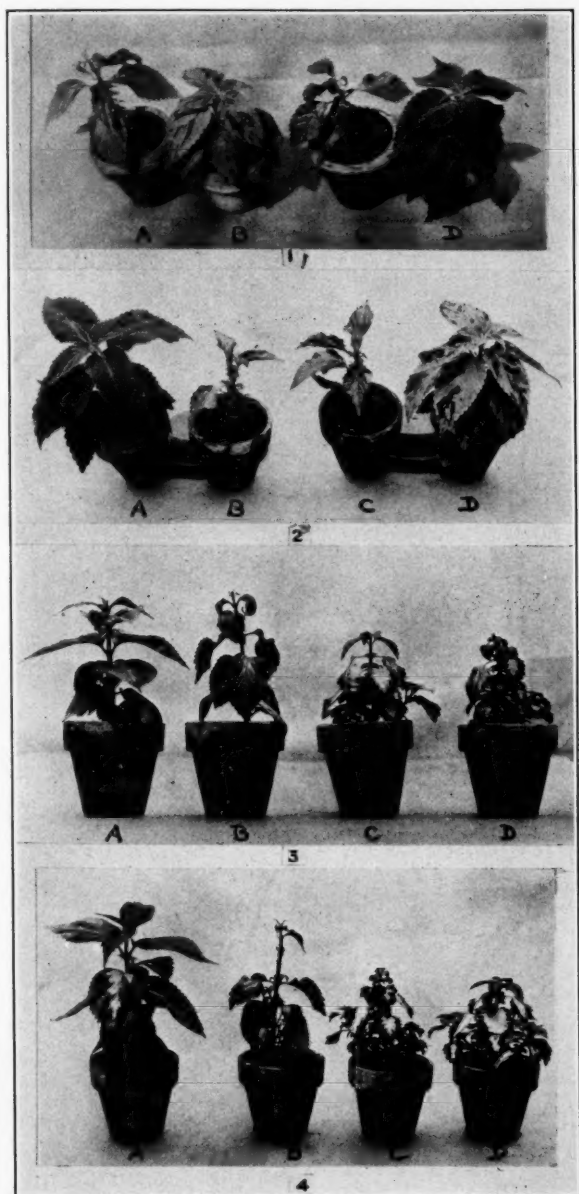
- A. Var. *Verschaaffeltii* not rayed.
- B. Var. *Verschaaffeltii* rayed for seven weeks at 100 inches from the light without a screen.
- C. Var. "Spotted Gem" rayed for seven weeks under the same conditions as in fig. 2 B.
- D. Var. "Spotted Gem" not rayed.

Fig. 3. *Coleus Blumei* var. "Defiance" and "Trailing Queen."

- A. Var. "Defiance" not rayed.
- B. Var. "Defiance" rayed for four weeks under the same conditions as the plants in fig. 1 A.
- C. Var. "Trailing Queen" not rayed.
- D. Var. "Trailing Queen" rayed for four weeks under the same conditions as fig. 1 A.

Fig. 4. *Coleus Blumei* var. "Defiance" and "Trailing Queen."

- A. Var. "Defiance" not rayed.
- B. Var. "Defiance" rayed for seven weeks under the same conditions as fig. 1 A.
- C. Var. "Trailing Queen" rayed for seven weeks under the same conditions as fig. 1 A.
- D. Var. "Trailing Queen" not rayed.



ELTINGE—EFFECT OF ULTRA-VIOLET RADIATION





EXPLANATION OF PLATE

PLATE 24 (SERIES II AND III)

Fig. 1. *Cucumis sativus*.

- A. Plant not rayed.
- B. Plant rayed for seven weeks at 100 inches, using a screen of quartz-lite glass.
- C. Plant rayed for seven weeks at 50 inches, using a screen of quartz-lite glass.
- D. Plant rayed for seven weeks at 100 inches, using a screen of vita glass.
- E. Plant rayed for seven weeks at 50 inches, using a screen of vita glass.

Fig. 2. *Ipomoea Batatas*.

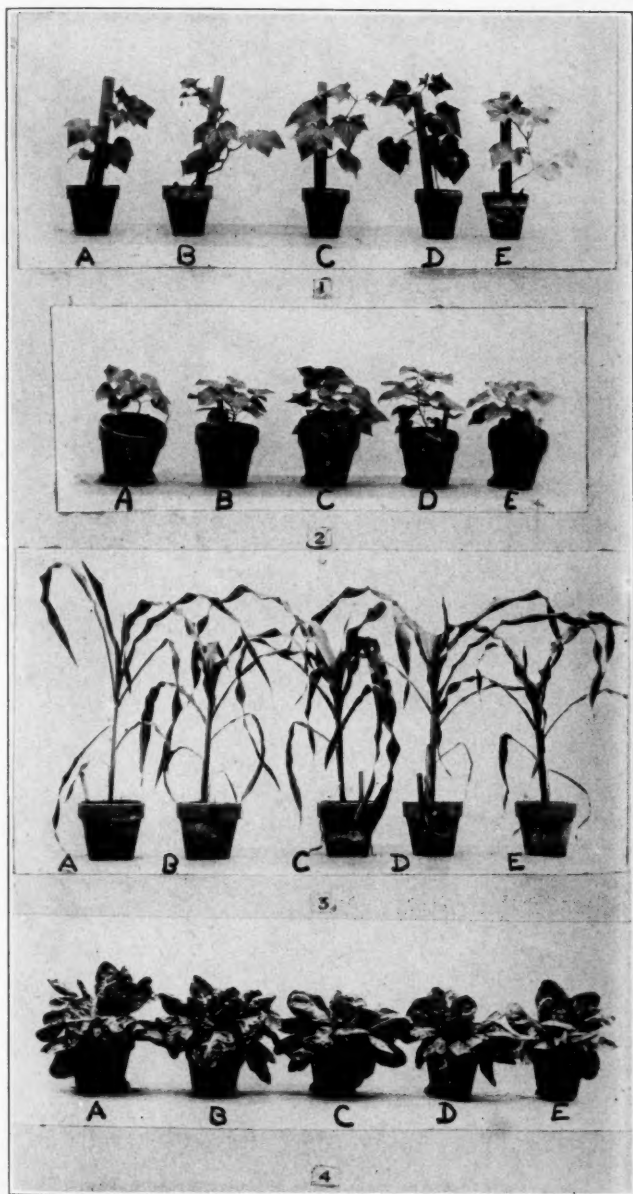
- A. Plant not rayed.
- B. Plant rayed for seven weeks at 100 inches, using a screen of quartz-lite glass.
- C. Plant rayed for seven weeks at 50 inches, using a screen of quartz-lite glass.
- D. Plant rayed for seven weeks at 100 inches, using a screen of vita glass.
- E. Plant rayed for seven weeks at 50 inches, using a screen of vita glass.

Fig. 3. *Zea Mays*.

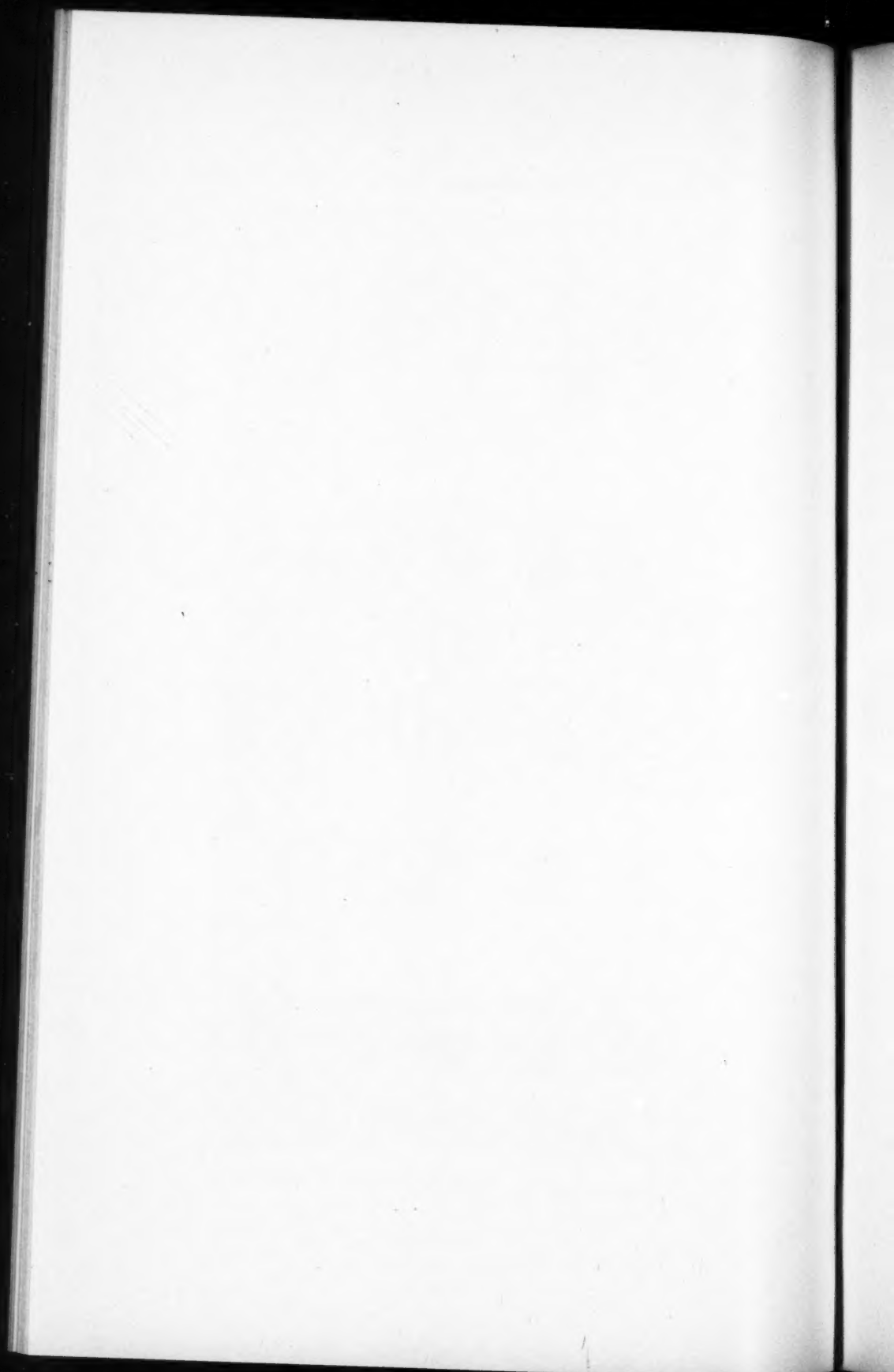
- A. Plant not rayed.
- B. Plant rayed for seven weeks at 100 inches, using a screen of quartz-lite glass.
- C. Plant rayed for seven weeks at 50 inches, using a screen of quartz-lite glass.
- D. Plant rayed for seven weeks at 100 inches, using a screen of vita glass.
- E. Plant rayed for seven weeks at 50 inches, using a screen of vita glass.

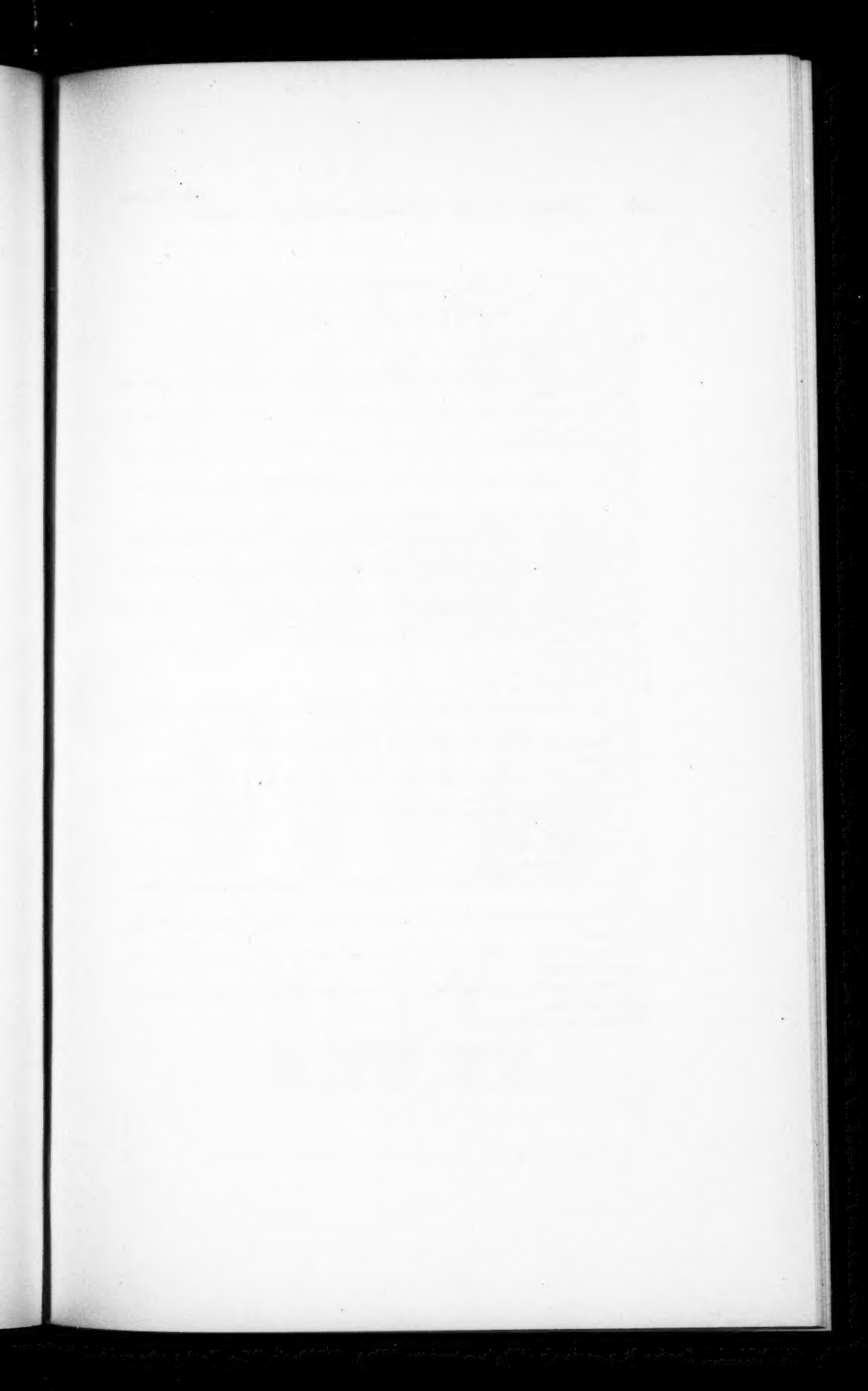
Fig. 4. *Lactuca sativa*.

- A. Plant not rayed.
- B. Plant rayed for seven weeks at 100 inches, using a screen of quartz-lite glass.
- C. Plant rayed for seven weeks at 50 inches, using a screen of quartz-lite glass.
- D. Plant rayed for seven weeks at 100 inches, using a screen of vita glass.
- E. Plant rayed for seven weeks at 50 inches, using a screen of vita glass.



ELTINGE—EFFECT OF ULTRA-VIOLET RADIATION





EXPLANATION OF PLATE

PLATE 25 (SERIES II AND III)

Fig. 1. *Raphanus sativus*.

- A. Plant not rayed.
- B. Plant rayed for seven weeks at 100 inches from the light, using a screen of quartz-lite glass.
- C. Plant rayed for seven weeks at 50 inches from the light, using a screen of quartz-lite glass.
- D. Plant rayed for seven weeks at 100 inches from the light, using a screen of vita glass.
- E. Plant rayed for seven weeks at 50 inches from the light, using a screen of vita glass.

Fig. 2. *Coleus Blumei* var. "Spotted Gem."

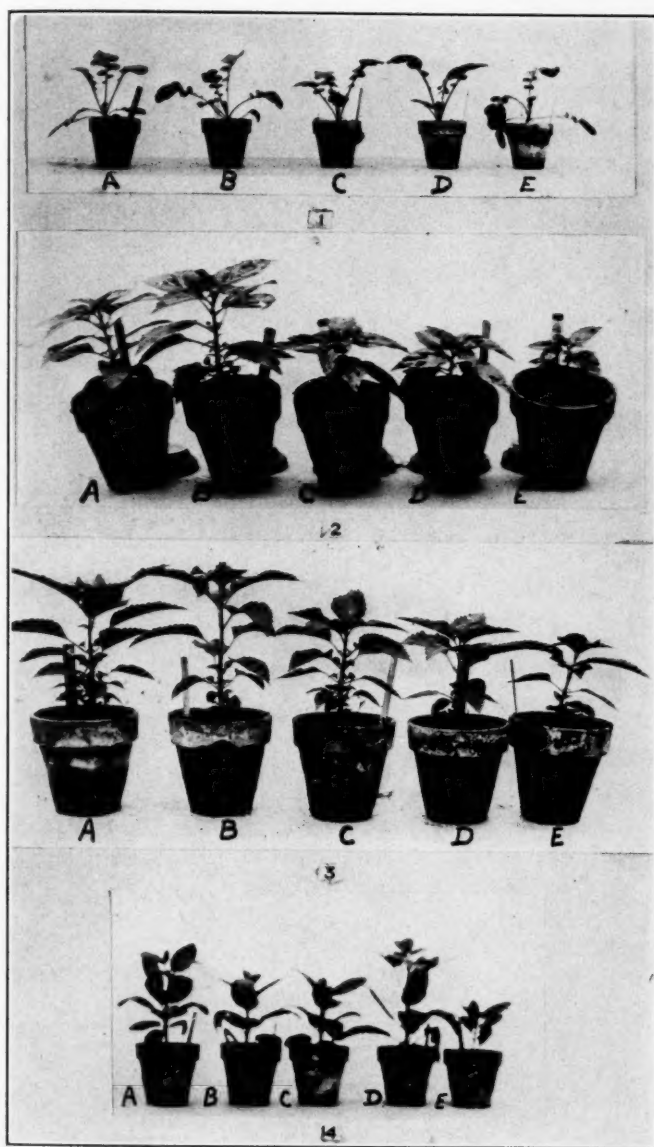
- A. Plant rayed for seven weeks at 50 inches from the light, using a screen of vita glass.
- B. Plant rayed for seven weeks at 100 inches from the light, using a screen of vita glass.
- C. Plant rayed for seven weeks at 50 inches from the light, using a screen of quartz-lite glass.
- D. Plant rayed for seven weeks at 100 inches from the light, using a screen of quartz-lite glass.
- E. Plant not rayed.

Fig. 3. *Coleus Blumei* var. *Verschaffeltii*.

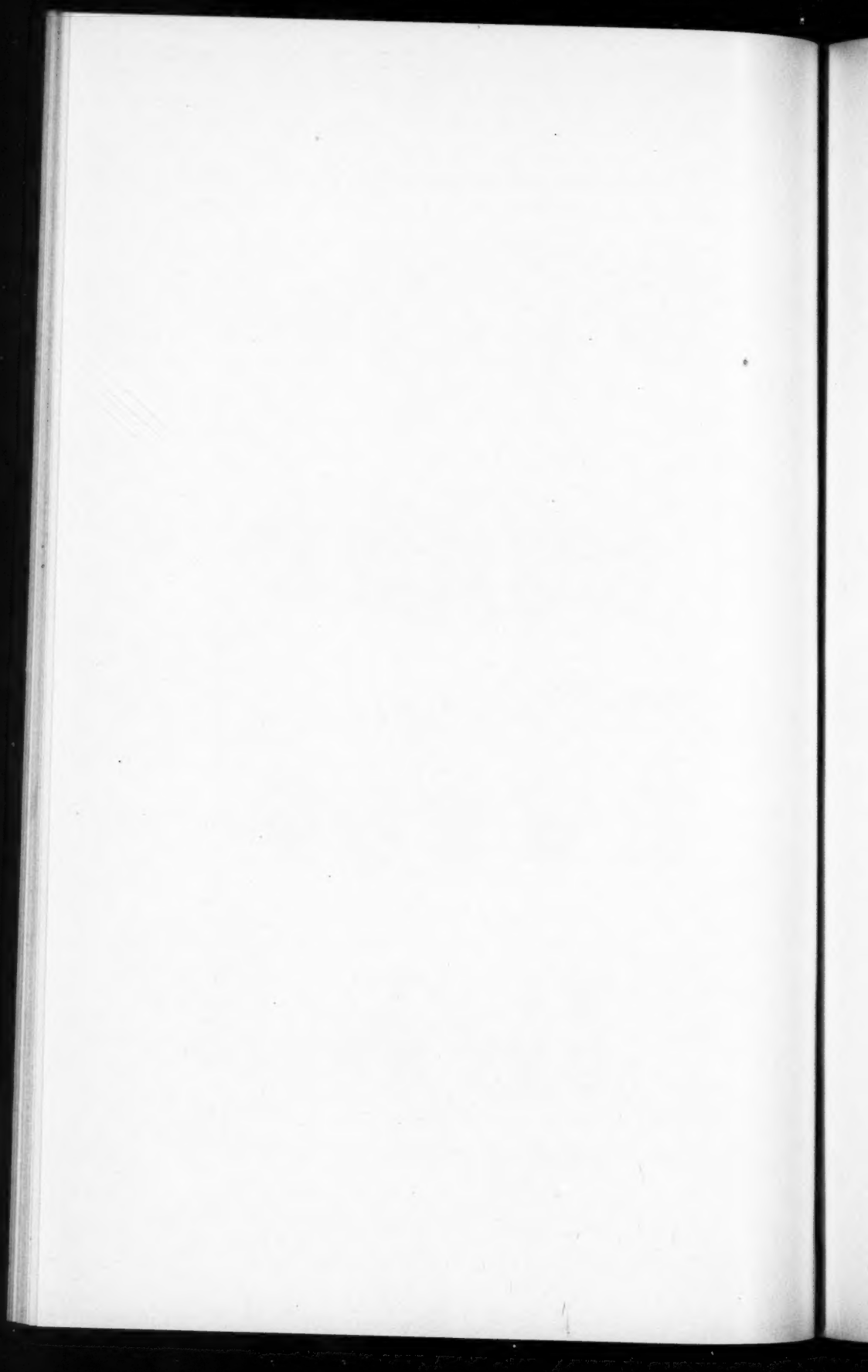
- A. Plant rayed for seven weeks at 50 inches from the light, using a screen of vita glass.
- B. Plant rayed for seven weeks at 100 inches from the light, using a screen of vita glass.
- C. Plant rayed for seven weeks at 50 inches from the light, using a screen of quartz-lite glass.
- D. Plant rayed for seven weeks at 100 inches from the light, using a screen of quartz-lite glass.
- E. Plant not rayed.

Fig. 4. *Bryophyllum pinnatum*.

- A. Plant rayed for seven weeks at 50 inches from the light, using a screen of vita glass.
- B. Plant rayed for seven weeks at 100 inches from the light, using a screen of vita glass.
- C. Plant rayed for seven weeks at 50 inches from the light, using a screen of quartz-lite glass.
- D. Plant rayed for seven weeks at 100 inches from the light, using a screen of quartz-lite glass.
- E. Plant not rayed.



ELTINGE—EFFECT OF ULTRA-VIOLET RADIATION



EXPLANATION OF PLATE

PLATE 26

Fig. 1. *Lactuca sativa* (fifteen leaves).

- A. Plant rayed for eight weeks at 100 inches from an unscreened lamp.
- B. Plant not rayed.

Fig. 2. *Coleus Blumei* var. *Verschaaffeltii*.

- A. Plant rayed for thirty seconds each day at 100 inches from an unscreened lamp.
- B. Plant not rayed.

Fig. 3. Leaves of *Coleus Blumei* var. *Verschaaffeltii*, with petioles paraffined.

- A. Leaf twelve hours after it had been rayed for $1\frac{1}{2}$ hours on each surface at thirty inches from an unscreened lamp.
- B. Leaf twelve hours after it had been rayed for $1\frac{1}{2}$ hours upon the upper surface at thirty inches from an unscreened lamp.
- C. Unrayed leaf after twelve hours.

Fig. 4. *Zea Mays*.

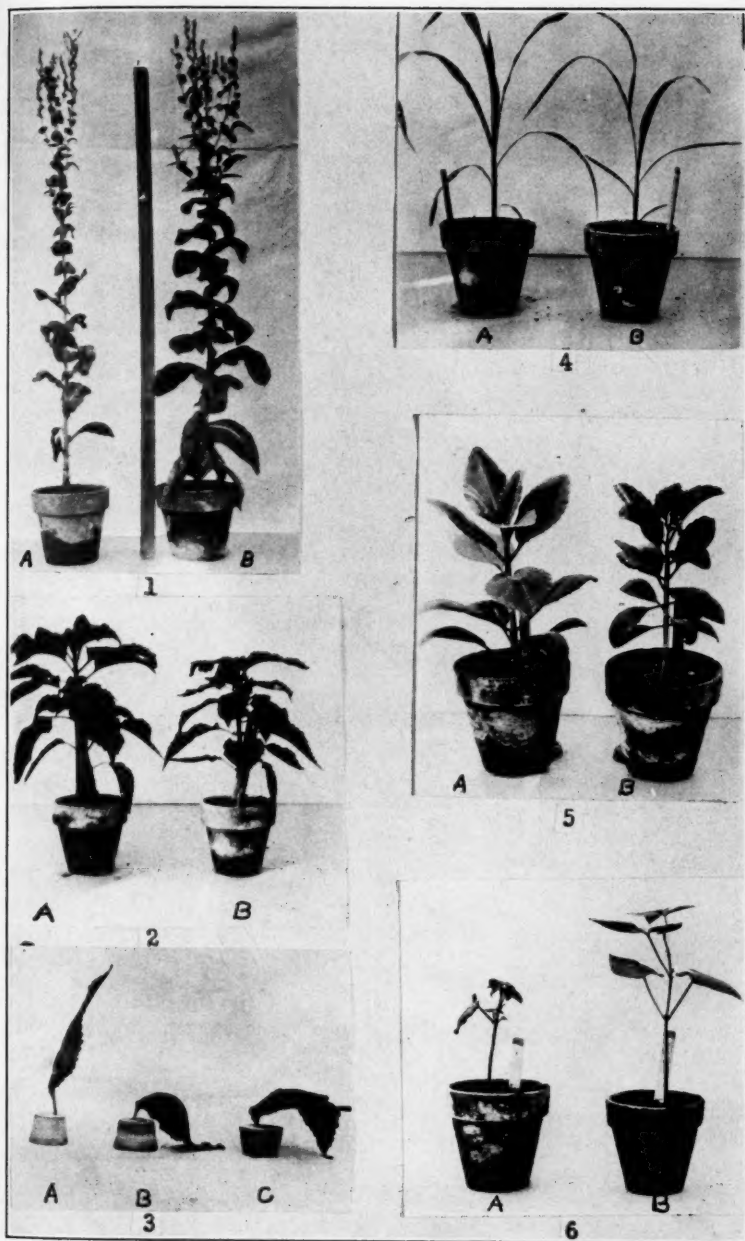
- A. Plant not rayed.
- B. Plant rayed for six weeks at 100 inches from an unscreened lamp.

Fig. 5. *Bryophyllum pinnatum*.

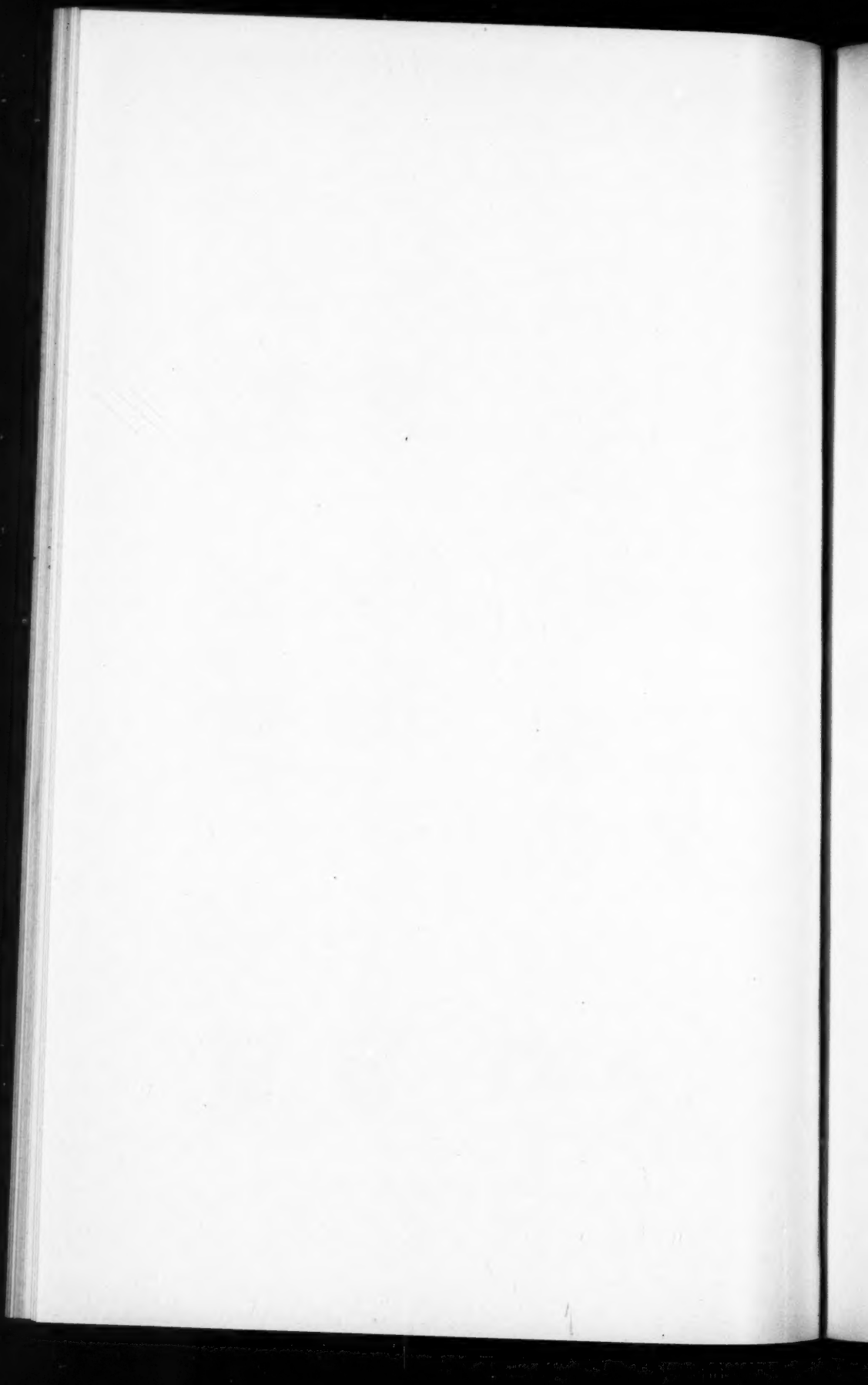
- A. Plant not rayed.
- B. Plant rayed for six weeks at 100 inches from an unscreened lamp.

Fig. 6. *Phaseolus vulgaris*.

- A. Plant rayed for four weeks at 100 inches from an unscreened lamp.
- B. Plant not rayed.



ELTINGE—EFFECT OF ULTRA-VIOLET RADIATION



EXPLANATION OF PLATE

PLATE 27

Camera-lucida drawings of equal magnification, using 4-mm. objective and 10 × eyepiece.

Fig. 1. Leaf of *Lactuca sativa* not rayed.

Fig. 2. Leaf of *Lactuca sativa* rayed for four weeks at 100 inches from the light without a screen.

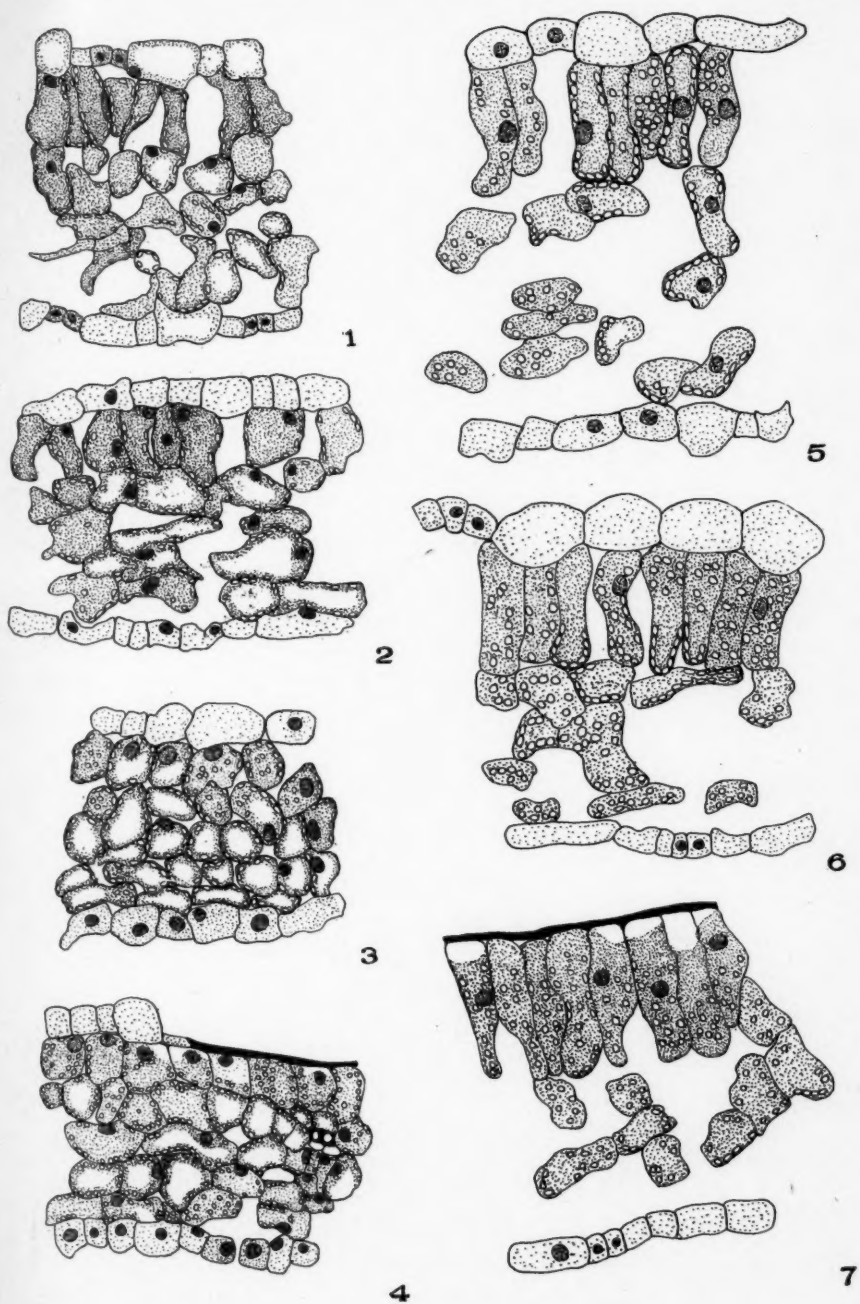
Fig. 3. Leaf of *Lactuca sativa* rayed for eight weeks at 100 inches from the light without a screen.

Fig. 4. Leaf of *Lactuca sativa* rayed for four weeks at 50 inches from the light without a screen.

Fig. 5. Leaf of *Nicotiana Tabacum* rayed for seven weeks at 100 inches from the light, using a screen of quartz-lite glass.

Fig. 6. Leaf of *Nicotiana Tabacum* not rayed.

Fig. 7. Leaf of *Nicotiana Tabacum* rayed for four weeks at 50 inches from the light without a screen.



ELTINGE—EFFECT OF ULTRA-VIOLET RADIATION



EXPLANATION OF PLATE

PLATE 28

Camera-lucida drawings of equal magnification, using 4-mm. objective and 10 X eyepiece.

Fig. 1. Leaf of *Phaseolus vulgaris* not rayed.

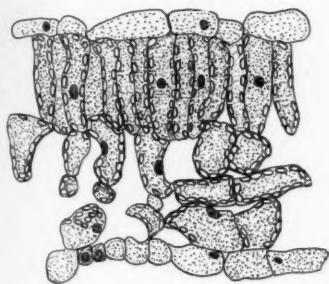
Fig. 2. Leaf of *Phaseolus vulgaris* rayed for four weeks at 100 inches from the light without a screen.

Fig. 3. Leaf of *Phaseolus vulgaris* rayed for seven weeks at 50 inches from the light, using a screen of quartz-lite glass.

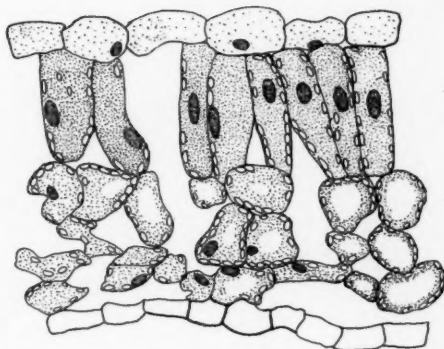
Fig. 4. Leaf of *Cucumis sativus* not rayed.

Fig. 5. Leaf of *Cucumis sativus* rayed for four weeks at 100 inches from the light without a screen.

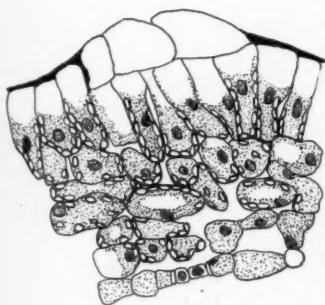
Fig. 6. Leaf of *Phaseolus vulgaris* rayed for seven weeks at 50 inches from the light, using a screen of vita glass.



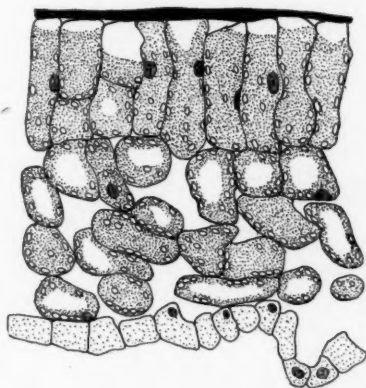
1



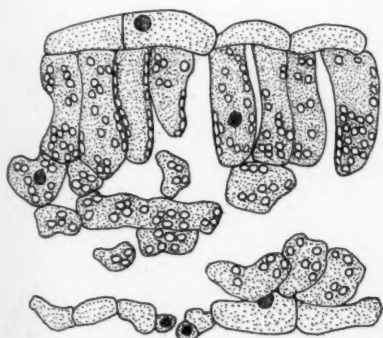
4



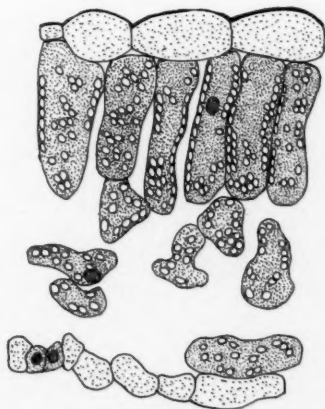
2



5

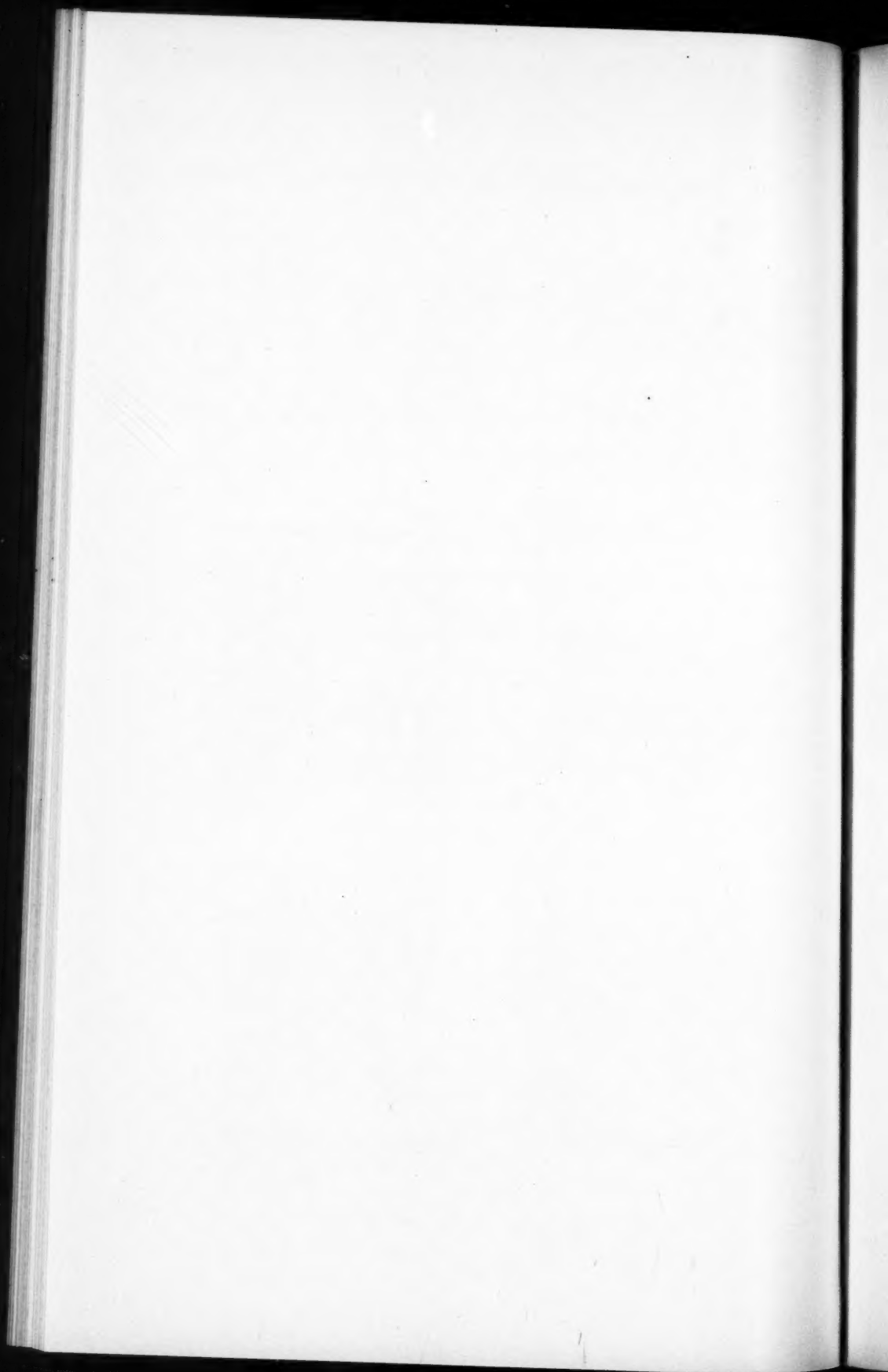


3



6

ELTINGE—EFFECT OF ULTRA-VIOLET RADIATION



EXPLANATION OF PLATE

PLATE 29

Camera-lucida drawings of equal magnification, using 4-mm. objective and 10 × eyepiece.

Fig. 1. Leaf of *Cucumis sativus* unrayed.

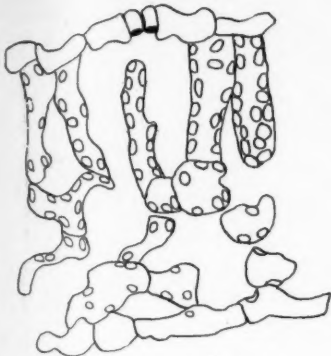
Fig. 2. Leaf of *Cucumis sativus* rayed for seven weeks at 100 inches from the light, using a screen of vita glass.

Fig. 3. Leaf of *Cucumis sativus* rayed for seven weeks at 100 inches from the light, using a screen of quartz-lite glass.

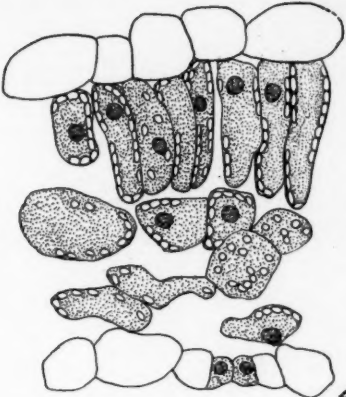
Fig. 4. Leaf of *Ipomoea Batatas* unrayed.

Fig. 5. Leaf of *Ipomoea Batatas* rayed for seven weeks at 100 inches from the light, using a screen of quartz-lite glass.

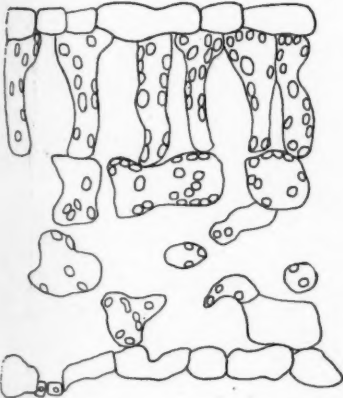
Fig. 6. Leaf of *Ipomoea Batatas* rayed at 50 inches from the light without a screen.



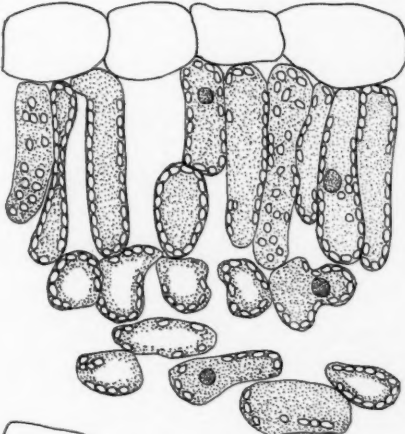
1



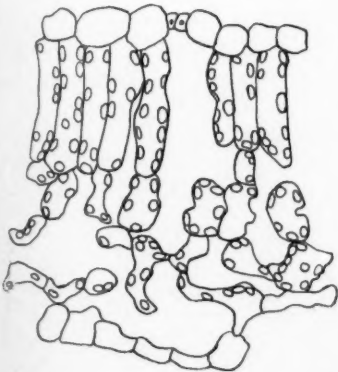
4



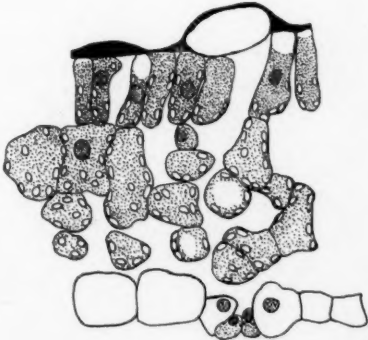
2



5



3



6

ELTINGE—EFFECT OF ULTRA-VIOLET RADIATION



EXPLANATION OF PLATE

PLATE 30

Camera-lucida drawings of equal magnification, using 4-mm. objective and 10 \times eyepiece.

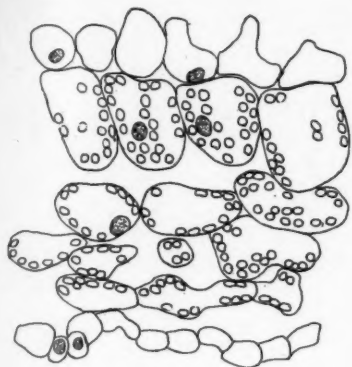
Fig. 1. Leaf of *Coleus Blumei* var. *Verschaffeltii* rayed for seven weeks at 50 inches from the light, using a screen of vita glass.

Fig. 2. Leaf of *Coleus Blumei* var. *Verschaffeltii* rayed for seven weeks at 100 inches from the light, using a screen of vita glass.

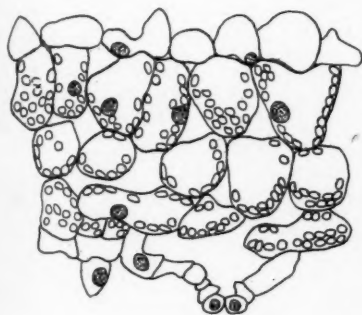
Fig. 3. Leaf of *Coleus Blumei* var. *Verschaffeltii* not rayed.

Fig. 4. Leaf of *Coleus Blumei* var. *Verschaffeltii* rayed for seven weeks at 50 inches from the light, using a screen of quartz-lite glass.

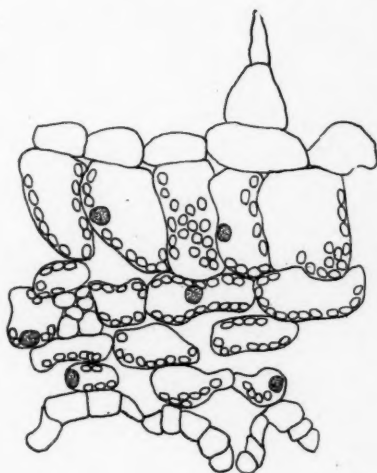
Fig. 5. Leaf of *Coleus Blumei* var. *Verschaffeltii* rayed for seven weeks at 100 inches from the light, using a screen of quartz-lite glass.



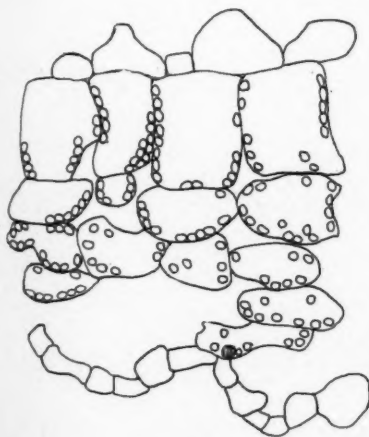
1



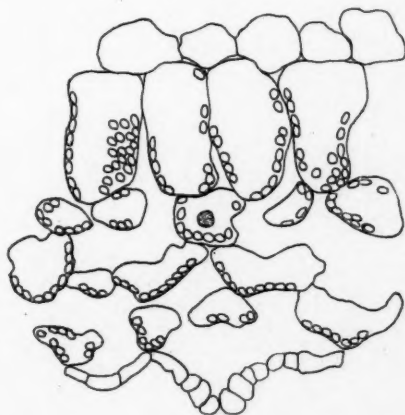
2



4

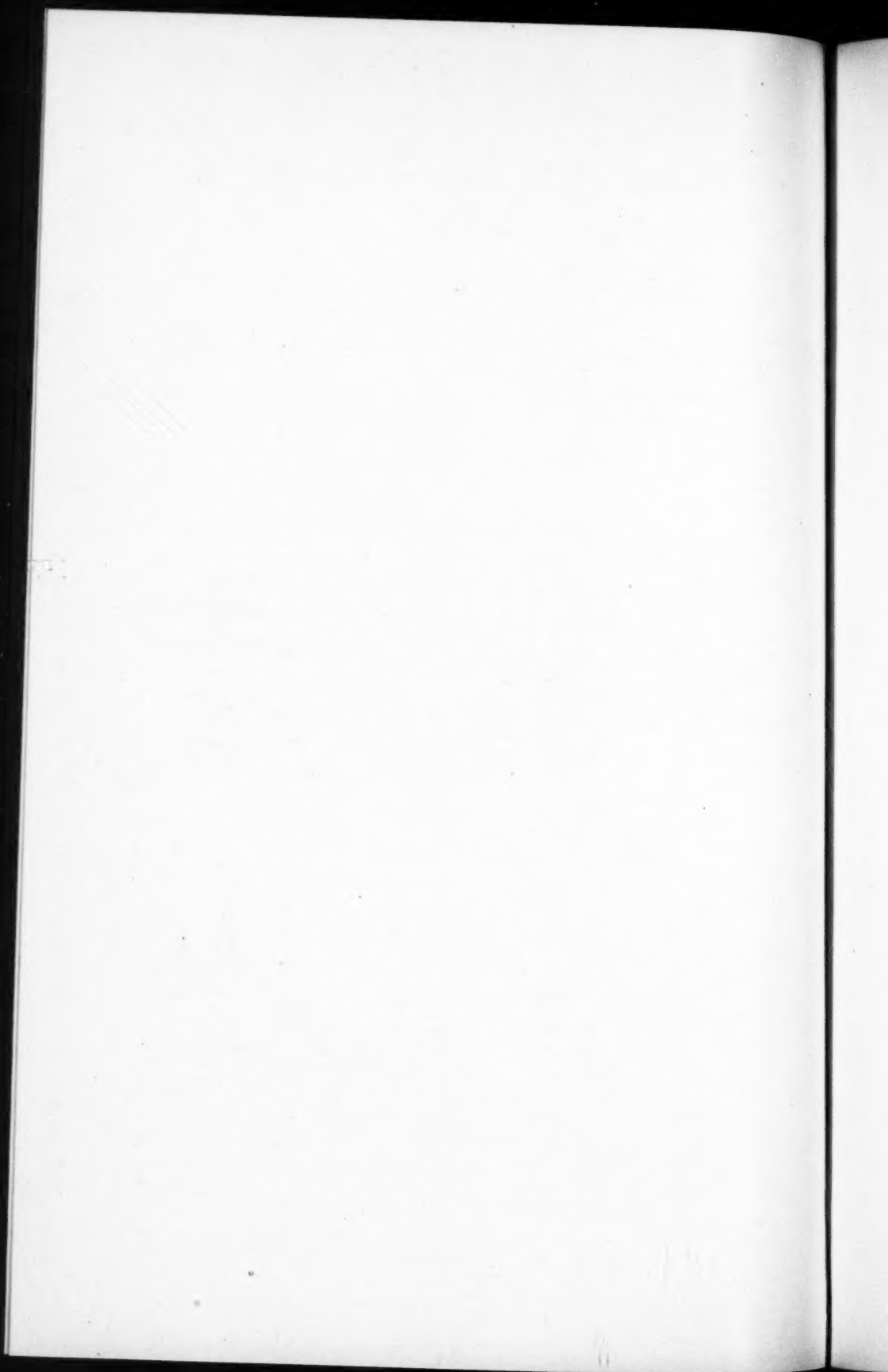


3



5

ELTINGE—EFFECT OF ULTRA-VIOLET RADIATION



EXPLANATION OF PLATE

PLATE 31

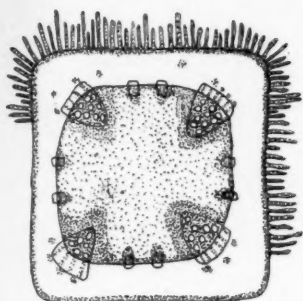
Fig. 1. Diagram of the cross-section of a stem of *Coleus Blumei*, the stippling indicating the normal distribution of red color.

Fig. 2. Diagram of the cross-section of a stem of *Coleus Blumei*, showing the distribution of red color at the end of the fifth raying at 50 inches from the light without a screen.

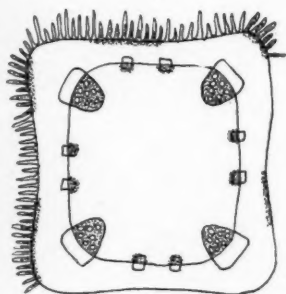
Fig. 3. Diagram of the cross-section of a stem of *Coleus Blumei*, showing the distribution of red color at the end of the tenth raying at 50 inches from the light without a screen.

Fig. 4. Cross-section of a leaf of *Coleus Blumei*, showing normal distribution of red color.

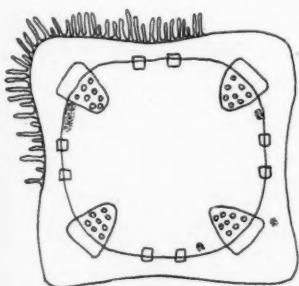
Fig. 5. Cross-section of a leaf of *Coleus Blumei*, showing the distribution of red color after ten rayings at 50 inches from the light without a screen. Drawn to the same scale as fig. 4.



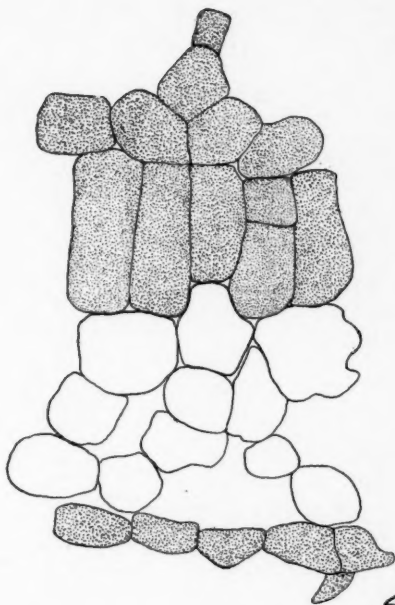
1



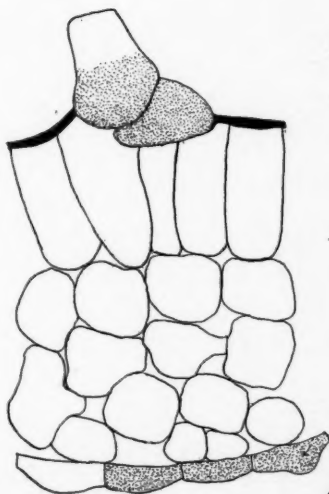
2



3



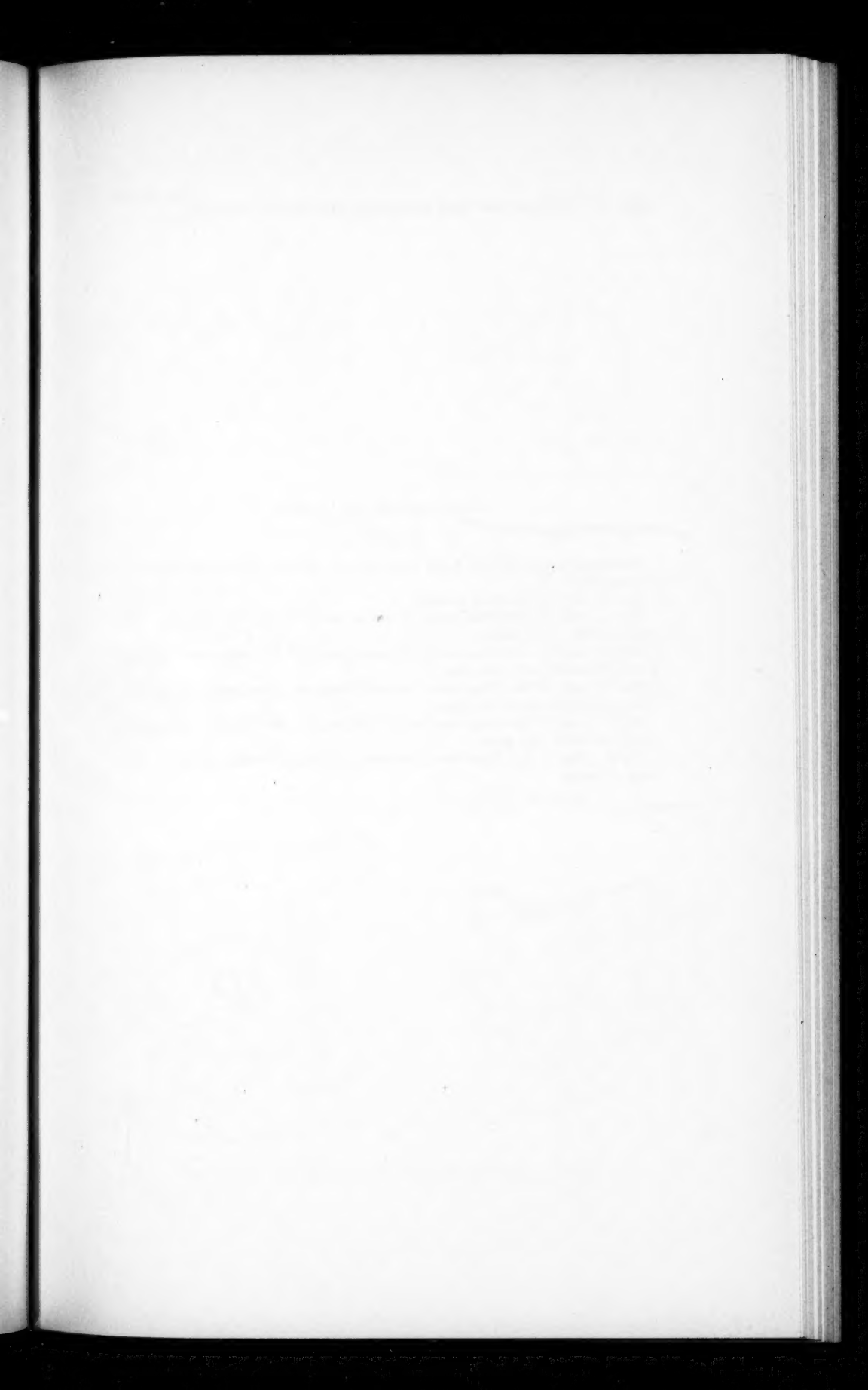
4



5

ELTINGE—EFFECT OF ULTRA-VIOLET RADIATION





EXPLANATION OF PLATE

PLATE 32

Camera-lucida drawings of equal magnification, using a 4-mm. objective and 10 × eyepiece.

Fig. 1. Leaf of *Zea Mays* unrayed.

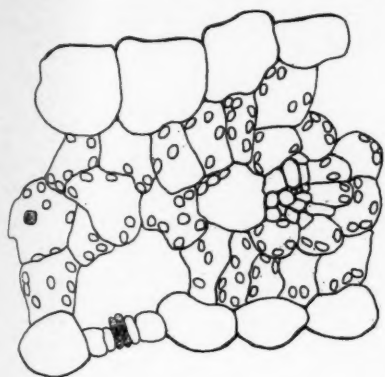
Fig. 2. Leaf of *Zea Mays* rayed for seven weeks at 50 inches from the light, using a screen of vita glass.

Fig. 3. Leaf of *Zea Mays* rayed for seven weeks at 50 inches from the light, using a screen of quartz-lite glass.

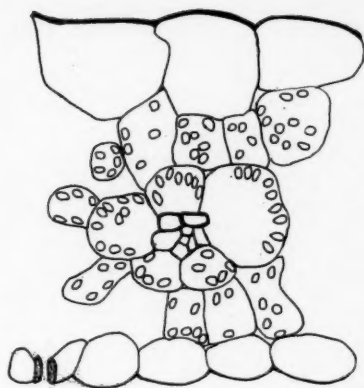
Fig. 4. Leaf of *Zea Mays* rayed for seven weeks at 100 inches from the light, using a screen of quartz-lite glass.

Fig. 5. Leaf of *Zea Mays* rayed at 100 inches from the light for seven weeks, using a screen of vita glass.

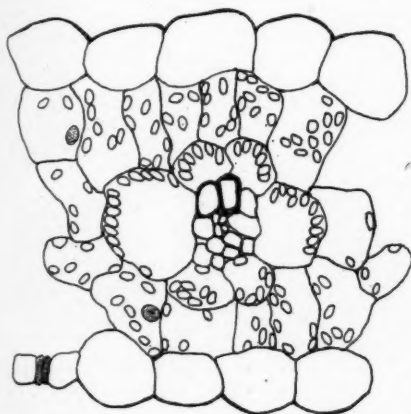
Fig. 6. Leaf of *Zea Mays* rayed for seven weeks at 100 inches from the light, using no screen.



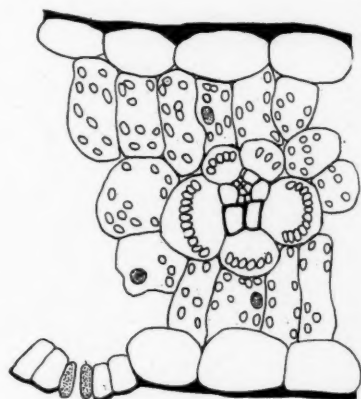
1



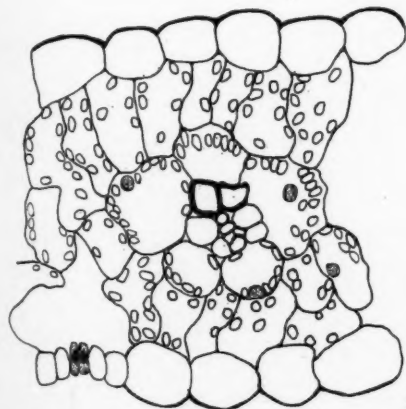
4



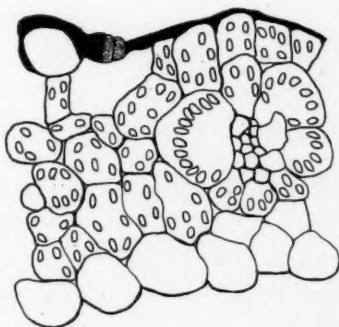
2



5



3



6

ELTINGE—EFFECT OF ULTRA-VIOLET RADIATION

EXPLANATION OF PLATE

PLATE 33

Camera-lucida drawings of equal magnification, using 16-mm. objective and 10 × eyepiece. Corresponding bundles were used in all cases (sixth bundle from the epidermis).

Fig. 1. Cross-section of a fibrovascular bundle of *Zea Mays* from an unrayed stem.

Fig. 2. Cross-section of a fibrovascular bundle of *Zea Mays* from a stem rayed for seven weeks at 50 inches from the light, using a screen of quartz-lite glass.

Fig. 3. Cross-section of a fibrovascular bundle of *Zea Mays* from a stem rayed for seven weeks at 50 inches from the light, using a screen of vita glass.

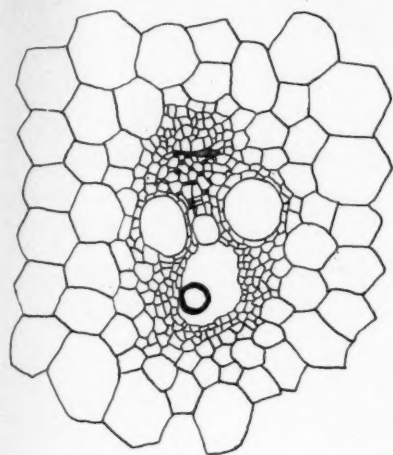
Fig. 4. Cross-section of a fibrovascular bundle of *Zea Mays* from a stem rayed for seven weeks at 100 inches from the light, using a screen of vita glass.

Fig. 5. The amount of cortex present in a stem of *Zea Mays* rayed for seven weeks at 50 inches from the light, using a screen of quartz-lite glass.

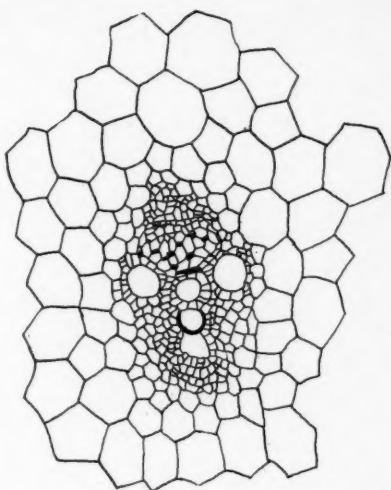
Fig. 6. The amount of cortex present in a stem of *Zea Mays* rayed for seven weeks at 50 inches from the light, using a screen of vita glass.

Fig. 7. The amount of cortex present in a stem of *Zea Mays* rayed for seven weeks at 100 inches from the light, using a screen of vita glass.

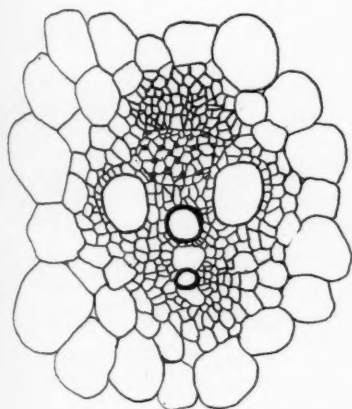
Fig. 8. The amount of cortex present in an unrayed stem of *Zea Mays*.



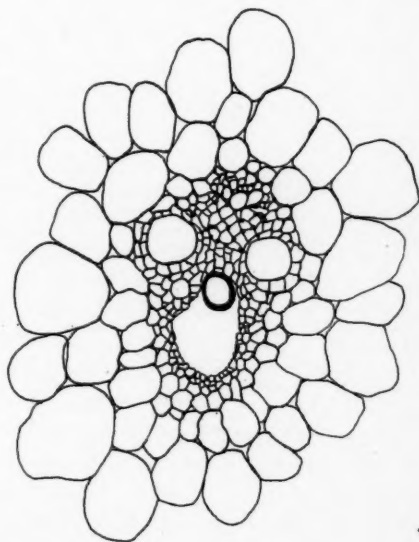
1



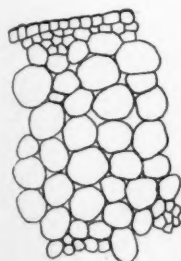
3



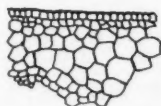
2



4



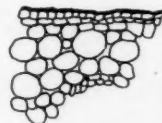
5



6



7



8

ELTINGE—EFFECT OF ULTRA-VIOLET RADIATION

